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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

IN RE APPLICATION OF: JOHN F.T. CONROY, M.E. POWER, AND P.M. NORRIS

Serial No. : 09/785,188 Art Unit: 1651
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Title : SOL-GEL BIOMATERIAL IMMOBILIZATION

Mail Stop Appeal Brief - Patents
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P.O. Box 1450
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REPLY BRIEF

Pursuant to 37 C.F.R. § 1.193(b)(1), Applicant responds to the Examiner's Answer dated March 28, 2005, as follows:

At page 6, line 3-8: In arguing that the rejection of claims 26 and 29 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that it would have been obvious to substitute vegetative yeast cells for the yeast spores found in Uo's gels "as suggested by Hino et al."

Hino suggests nothing of the sort. Hino makes no mention of vegetative yeast cells whatsoever. A rejection that is based on the use of vegetative cells is not based on the scope and content of Uo and Hino, but rather on a speculative combination of elements not found in either reference.

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At page 6, line 8-13: In arguing that the rejection of claims 26 and 29 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that using vegetative cells in Uo's matrices would have been expected to simplify immobilization. In particular, the Answer contends that if vegetative cells were immobilized directly, they would not have to be converted to spores prior to immobilization and then back to the vegetative state for use. As best understood, this contention is to be a motivation for combining Uo and Hino.

Rather than providing a motivation for combining Uo and Hino, this contention further illustrates that one of ordinary skill would not immobilize vegetative cells in Uo's matrices with a reasonable expectation of success. In particular, under the logic of this contention, Uo went out of his way to avoid immobilizing vegetative cells. In doing so, he intentionally performed additional steps and complicated his immobilization process. Despite the urgings of the Examiner's Answer to the contrary, Uo explicitly identifies why he immobilized yeast spores. Namely, according to Section 2.2 of Uo, page 427, yeast spores were immobilized for their durability to organic solvents.

The Examiner's Answer now attempts to discard this express teaching of Uo regarding the desirability of yeast spore immobilants. *The Examiner's Answer assumes that it knows how to immobilize cells better than Uo himself,* dismissing Uo's intentional selection of yeast spores as unduly complicated. No basis founded in the art of record for this "obvious" improvement to Uo's process is presented. Rather, the Examiner's Answer bases an obviousness rejection on the assumption that Uo is intentionally and unduly complicating his immobilization process without any justification for the assumption founded in the prior art.

At page 6, line 14-21 and page 8, lines 6-23: In arguing that the rejection of claims 26 and 29 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that the claims are deficient for not requiring that immobilized cells have a certain amount of activity after immobilization.

However, Applicant submits that there is no motivation to combine Uo and Hino without the immobilized cells having a certain amount of activity. The rejections of claims 26 and 29 are obviousness rejections under 35 U.S.C. § 103(a). Some motivation to combine the references must be present to establish a *prima facie* case of obviousness. *If the combination of Uo and Hino does not result in immobilized cells having a certain amount of activity, then there is no motivation for one of ordinary skill to combine Uo and Hino* in the manner suggested. In particular, there is no reason to believe that one of ordinary skill would select Uo's gelation solution to inactivate Hino's immobilants in a solid matrix.

Further, it is self-evident that both Uo and Hino expressly teach away from inactivating immobilants. Forming a gel with immobilants that lack a certain level of activity directly contradicts the teachings of both Uo and Hino. Therefore, as a "useful general rule," Uo and Hino cannot serve to create a *prima facie* case of obviousness where inactivated immobilants result from the combination. No reason for discarding this useful general rule has been provided.

At page 7, line 3–10: In arguing that the rejection of claims 26 and 29 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that it is within the level of ordinary skill in the art to exchange cell durability for simplification of the immobilization process.

The fact that one of ordinary skill in the art is *capable* of exchanging cell durability for simplification of the immobilization process is tangential to the issue of whether or not a *prima facie* case of obviousness has been established.

Rather, a fundamental issue is whether or not one or ordinary skill would be *motivated* to exchange cell durability for simplification of the immobilization process. Uo and Hino clearly teach away from the inactivation of immobilants, and Uo leads away from the exact combination proposed by the Examiner's answer. Block at least suggests that one of ordinary skill would not have a reasonable expectation of success with the combination.

Nevertheless, the Examiner's answer insists on finding a motivation that disregards every reference of record. Absent some basis founded in the prior art that one of ordinary skill would indeed find the immobilization of inactivated immobilants attractive, the rejection cannot be maintained.

At page 7, line 15-29: In arguing that the rejection of claim 28 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that it would have been obvious to substitute bacterial spores for the yeast spores found in Uo's gels.

Neither Uo nor Hino make any mention of bacterial spores whatsoever. A rejection that is based on bacterial spores is not based on the scope and content of Uo and Hino, but rather on a speculative combination of elements not found in either reference.

At page 9, line 1-9: Given that both claims 26 and 29 require macroporous gels, in arguing that the rejections of claims 26 and 29 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that Hino suggests that a macroporous silica gel

like Uo's can be obtained with an acceptable level of inactivation. **At page 10, line 17-22**, the Examiner's Answer contends that it would be obvious to omit methanol from Uo's process for immobilizing cells and yet a macroporous gel would still be obtained.

These contentions represent nothing more than unsupported armchair chemistry. A macroporous gel like Uo's without methanol is neither described nor suggested by Uo. A rejection that is based on a macroporous gel without methanol is not based on the scope and content of Uo and Hino, but rather on a speculative combination of elements not found within the scope and content of the references.

Further, every one of Hino's gels that immobilizes cells is explicitly excluded from being macroporous. Please see the Appeal Brief for a discussion of this point. Hino thus does not suggest that macroporous silica gels with an acceptable level of inactivation can be obtained.

Further, every recipe for macroporous silica gels in the art of record (including Uo's) requires toxic gelation conditions. Please see the paragraph bridging pages 9-10 of the Appeal Brief. Thus, the scope and content of the prior art suggests the exact opposite conclusion, namely, that macroporous silica gel like Uo's cannot be obtained without an unacceptable level of inactivation.

An obviousness rejection must be based on the scope and content of the prior art. The continued departure of the rejections from what is actually described in the art and reliance upon what the Examiner's Answer feels is obvious or possible is erroneous. *In re Lee,* 277 F.3d 1338 (Fed. Cir. 2002).

At page 9, line 19 – page 10, line 4: In arguing that the rejections of claims 26 and 29 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that Uo does

not expose yeast spores to gelation solution for one day. In particular, the Examiner's Answer contends that after gelation, the spores are not exposed to gelation solution.

While agreeing with the assertion that Uo's yeast spores are not exposed to *pure* gelation solution for over one day, Applicant respectfully disagrees with the contention that, after gelation, the spores are no longer exposed to gelation solution. In Uo and every other reference of record, there is no instantaneous removal of the gelation solution from the pores of a gel after gelation. For example, at col. 12, line 2-6, Hino describes that the liquid component of lyogels is to be removed from the lyogels by ventilation drying to produce xerogels.

Uo's yeast spore are thus exposed to pure gelation solution during mixing, during the gelation reaction, and during any aging of the gel after gelation. Once gelation and any aging is complete, an alternative method called solvent exchange can be used to exchange the gelation solution with another solution. Uo uses such a solvent exchange process when he describes that the gels are crushed and soaked in sterilized water for one day at room temperature. During this day, the sterilized water penetrates the pores of the gel, gradually diluting the gelation solution in the pores until a uniform distribution of the mixture of sterilized water and gelation solution remains.

Thus applicant submits that Uo's yeast spores are exposed to pure gelation solution during mixing, during the gelation reaction, and during any aging of the gel after gelation. Uo's yeast spores are exposed to increasingly-diluted gelation solution during the day-long solvent exchange.

At page 10, line 4-22: In arguing that the rejections of claims 26 and 29 as obvious over Uo and Hino should be maintained, the Examiner's Answer speculates, without support, on a

number of points. Together, these points amount to an assertion that Applicant has not proven that Uo's gelation solutions are toxic to Hino's immobilants. In particular, the Examiner's Answer speculates that TMOS and PEG *could* lessen the microbicidal affect of Uo's gelation solutions beyond that of otherwise pure methanol/water mixtures, and that the 45-55 Vol.% methanol in Uo's gelation solutions *could* be substantially less toxic than the 65 Vol.% methanol disclosed as toxic to *Staphylococcus aureus* and *Escherichia coli* in under 1 minute.

As to the gist of the unsupported speculation (i.e., that Applicant has not proven that Uo's gelation solutions are toxic to Hino's immobilants), it is well established that the burden of establishing a *prima facie* case of obviousness lies with the Office. Applicant is therefore not required to prove, at any evidentiary standard, that a particular combination proposed by the Office will not work. Rather, the Office is required to establish that one of ordinary skill would be motivated to carry out a proposed combination with a reasonable expectation of success.

That Uo, Hino, and Block at least suggest that one of ordinary skill would not have a reasonable expectation of success with the proposed combination does nothing to remove this burden from the Office. Rather, these references *must be considered* by the Office when attempting to establish the expectations of one of ordinary skill. The Office cannot dispense with these references simply by arguing that Applicant has not met an evidentiary burden of proving that a proposed combination will not, in fact, work. Rather, the Office must establish that one of ordinary skill would have a reasonable expectation of success with the proposed combination.

Turning to the individual points, the unsupported speculation that TMOS and PEG *could* lessen the microbicidal affect of Uo's gelation solutions and that the 45-55 Vol.% methanol in Uo's gelation solutions *could* be substantially less toxic than the 65 Vol.% methanol insufficient to establish that one of ordinary skill would reasonably expect success with the proposed

combination. Perhaps if the Office could establish that the presence of TMOS and PEG would in fact lessen the microbicidal affect of Uo's gelation solutions beyond that of otherwise pure methanol/water mixtures (and overcome the express teaching of Uo to the contrary), one of ordinary skill might have been provided with a reasonable expectation of success. Perhaps if the Office could establish that 45-55 Vol.% methanol is in fact substantially less toxic than the 65 Vol.% methanol (and overcome the express teaching of Uo to the contrary), one of ordinary skill might have been provided with a reasonable expectation of success. However, no such showing has been made and a prima facie case of obviousness has not been established.

At page 11, line 1 – page 12, line 2 and page 12, line 12-16: In arguing that the rejections of claims 26 and 29 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that Hino's solvent casting in isopropanol of gels that retain 61% of their activity is evidence that Hino's vegetative cells can be contacted with Uo's methanol and retain their activity. The Exmainer's Answer thus contends that Hino's cells are exceptions to the general susceptibility of vegetative cells to alcohols investigated over centuries and described in Block.

Applicant respectfully disagrees. Hino's solvent casting involves the extrusion of Hino's cell-containing gelation solutions into isopropanol. As the solutions contact the isopropanol, they gel, retaining the gelation solution in their pores. Once again, there is no instantaneous removal of the gelation solution from the pores of a gel after gelation. Normally, if the gels are allowed to soak in the casting solution (here, isopropanol), the casting solution will penetrate the gel over time as it solvent exchanges with the gelation solution. However, Hino goes out of his way to avoid allowing isopropanol to reach the interior of the gel. In particular, Hino freeze

dries the cast gels immediately. Such a freeze drying of the gels freezes the solvents, stopping the solvent exchange and allowing the cells on the interior of the gel to remain in contact with gelation solution.

Thus, Hino also teaches that contact between cells and isopropanol is to be limited, and that even contact limited by immediate freeze drying inactivates cells. This is not surprising given the teachings regarding the toxicity of isopropanol found in Block cited in the Appeal Brief. Hino's cells thus are not exceptions to the general susceptibility of vegetative cells to alcohols described in Block.

At page 12, line 6-7: In arguing that the rejections of claims 26 and 29 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that it is Uo's PEG and not Uo's methanol that forms macropores in Uo's gels.

There is no support for this contention founded in the prior art. Indeed, contrary examples can be found. For example, Hino uses PEG and yet the majority of his gels are expressly precluded from including macropores. There is nothing to suggest that Uo's and Hino's chemistry is as simplistic as "PEG forms macropores, methanol doesn't."

Moreover, this contention ignores the other references of record in the application. Every recipe for macroporous silica gels (including Uo's) requires toxic gelation conditions. The Examiner's answer improperly disregards these references and instead relies upon unsupported speculation (i.e., "Uo's PEG forms macropores, Uo's methanol doesn't").

At page 12, line 10-12: In arguing that the rejections of claims 26 and 29 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that "there is

inadequate evidence to establish that the methanol of Uo et al. is critical to obtaining macropores."

However Uo neither describes nor suggests a methanol-free macroporous recipe. A rejection that is based on a speculative modification of Uo's macroporous recipe to exclude methanol is not based on the scope and content of Uo and Hino, but rather on a speculative combination of elements not found in Uo and Hino.

Further, Applicant is no burden to make this proof. The burden of establishing a *prima facie* of obviousness lies with the Office. The Office cannot dispense with this burden simply by arguing that Applicant has not met an evidentiary burden of proving that a proposed combination will not, in fact, work.

At page 14, line 7-8: In arguing that the rejection of claim 28 as obvious over Uo and Hino should be maintained, the Examiner's Answer contends that there is no "substantial difference" between gels containing macropores and gels without macropores.

Applicant respectfully submits that any reading of an express limitation out of the claims during examination is improper. Further, this statement is contradicted by the references of record such as Kajihara and Nakanishi that intentionally develop macroporous recipes.

At page 14, line 18-21: In arguing that the rejection of claim 28 as obvious over Uo and Hino should be maintained, the Examiner's Answer is understood to contend that since Uo does not investigate the experimental relationship between pore size and methanol content that methanol can be eliminated from Uo's gelation solution.

Applicant respectfully disagrees. The mere fact that Uo did or did not chose to investigate the dependence of pore size on an experimental variable does not suggest that the uninvestigated variable can be eliminated. For example, Uo also did not investigate the dependence of pore size on catalyst concentration, precursor concentration, temperature, and time. By the logic of the Examiner's Answer, these variables could also be "eliminated" without any effect on pore size.

Once again, a rejection must be based on the scope and content of the prior art. Uo's gelation solution absent methanol is not found in the cited references. A rejection cannot be based on unsupported armchair chemistry.

At page 16, line 4-7: In arguing that the rejection of claim 15 as obvious over Uo, Hino, Klein, and Rao should be maintained, the Examiner's Answer is understood to contend that since Uo's robust yeast spores can be immobilized in 45-55 Vol.% methanol solutions, one of ordinary skill would be motivated to immobilize Uo's yeast spores in an approximately 65 Vol.% ethanol solution with increased toxicity, increased temperature, and an increased concentration of organic solvent. **At page 16, line 4-7**, the Examiner's Answer contends that one of ordinary skill would be motivated to ignore the increased toxicity, increased temperature, and an increased concentration of organic solvents so as to achieve more complete hydrolysis and condensation, an increased rate of hydrolysis, and an increased surface area.

These contentions ignore the requirement that the motivation to combine must have a reasonable expectation of success. Even if one assumes that more complete hydrolysis and condensation, an increased rate of hydrolysis, and an increased surface area are factors that would motivate one of ordinary skill to deviate from the ratios of moles water to moles hydroxyl

metallate described in Uo and Hino, the use of the increased hydrolysis ratios must nevertheless provide a reasonable expectation of success.

The reasonable expectation of success is an element of a *prima facie* case of obviousness and the Office bears the burden of proof that the expectation is reasonable. Bald speculation that Uo's yeast spores would be expected to be resistant to gelation solutions with increased toxicity is insufficient. This expectation of success must be founded in the teachings of the prior art and not unsupported conclusions or an assertion that an evidentiary burden of proof away from the combination has not been met by the Applicant.

Further, Applicant has provided evidence that one of ordinary skill would expect nothing but increased inactivation with the proposed combination. Both Hino and Uo teach away from the inactivation of immobilants, as a general rule disqualifying their use as references to establish the proposed combination.

At page 16, line 7-11: In arguing that the rejection of claim 15 as obvious over Uo, Hino, Klein, and Rao should be maintained, the Examiner's Answer contends that it would be obvious to use a sol with a reduced amount of solvent so as to not to kill an undue number of cells.

None of the cited references describe or suggest a sol with the reduced amount of solvent suggested by the Examiner's Answer. A rejection that is based on a sol with the reduced amount of solvent is not based on the scope and content of the prior art, but rather on a speculative combination of elements.

Further, claim 15 is directed to a sol containing a ratio of 25 or more moles water for each mole hydroxyl metallate. Assuming that the Examiner's Answer is not contending that the

amount of aqueous solvent should be reduced (e.g., outside the claimed range), the Examiner's Answer is understood to contend that the amount of organic solvent should be reduced. However, the only reference that discusses a ratio of water/hydroxyl metallate within the claimed range (i.e., Klein), describes that additional ethanol is necessary to permit solubility of the increased water in the sol solution. *See* Klein, page 34, last sentence of the second paragraph.

The Examiner's Answer has provided no guidance as to why or how this teaching is to be ignored. Not only does the Examiner's Answer propose a combination that directly contradicts the express teachings of Klein, it offers no guidance as to how the physical reality of insolubility of solvents is to be overcome. *The Examiner's Answer assumes that it knows how to handle high hydrolysis ratios better than the references of record* and dismissed their express teachings as unnecessary. Moreover, the constraints of physical reality identified in Klein are ignored by the Examiner's Answer in forming the speculative combination of elements.

At page 16, line 11-13: In arguing that the rejection of claim 15 as obvious over Uo, Hino, Klein, and Rao should be maintained, the Examiner's Answer contends that that the claims are deficient for not requiring that immobilized cells have a certain amount of activity after immobilization.

Applicant submits that there is no motivation to combine Uo, Hino, Klein, and Rao without the immobilants having a certain amount of activity. In particular, there is no reason to believe that one of ordinary skill would select Klein's gelation solution to inactivate Hino's or Uo's immobilants.

Moreover, it is self-evident that both Uo and Hino expressly teach away from inactivating immobilants. Therefore, as a "useful general rule," Uo and Hino cannot serve to create a *prima*

facie case of obviousness where inactivated immobilants result from the combination. No reason for discarding this useful general rule has been provided.

For these reasons, and the reasons stated in the Appeal Brief, Applicant submits that the final rejection should be reversed.

At page 9, line 10-13 suggests that Chapter 12 of the publication entitled "Disinfection, Sterilization, and Preservation" (S.S. Block, *Ed.*) is not available. For the sake of insuring that the publication is available to the Board, a copy is submitted herewith.

No fee is believed to be due.

Respectfully submitted,

Date: 5/26/05

John F. Conroy

Date: May 23/05

Mary E. Power

Date: May 24, 2005

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Disinfection, Sterilization, and Preservation

Fifth Edition

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CHAPTER 12

Alcohols

Yosef Ali, Michael J. Dolan, Eleanor J. Fendler, and Elaine L. Larson

The term *alcohol* is derived from the Arabic *al kohl,* a term for the antimony sulfide used by ancient Egyptians to treat eye infections in newborns. The alchemist Paracelsus in the Middle Ages applied the name *al-kool* to the antiseptic properties of wine. Alcohol is perhaps the oldest of antiseptic agents, being recommended for wound treatment by Claudius Galen (131–201 AD) and Guy de Chauliac (1363). Beck (1990) reviewed the history of alcohol use as an antiseptic.

The first scientific investigation of the antimicrobial properties of alcohol was likely the ethanol studies of Buchholtz (1875), which suggested possible antiseptic use. Subsequently, Koch and Koch (1881) concluded that ethanol was ineffective as an antiseptic based on his work with anthrax spores. Nealton (Beck, 1984) first applied alcohol to preoperative skin disinfection in the nineteenth century. Fürbringer recommended alcohol as a hand disinfectant for surgeons in 1888 (Rotter, 1996b).

In many parts of Europe, the use of alcohols has remained the standard for hand and skin antisepsis since the early part of this century (Rotter, 1996b). Prevailing practices in the United States, however, have cycled over time. Ethanol was a preferred skin antiseptic in the 1930s. Price (1938a, 1939, 1950a,b, 1951, 1959) demonstrated the antiseptic performance of alcohol in a series of reports from 1938 through 1950. His survey of 1948 (Price, 1950) indicated 64% of U.S. hospitals used ethanol for skin disinfection. By 1965, this rate had declined to 18% (King and Zimmerman, 1965), and by 1992 alcohols were seldom used for skin and especially hand antisepsis.

Today, alcohols are not primary skin antiseptics in North America, having been replaced by antiseptic preparations containing various active ingredients, including iodophors, chlorhexidine gluconate, phenolic derivatives [p-chloro-m-xylenol (PCMX), triclosan], and quarternary ammonium compounds (benzyl/benzalkonium chlorides). Some reversal of this trend began in the late 1980s with the introduction of alcohol-based "hand sanitizers." These products, which contained 60% to 70% ethanol, isopropanol, or mixtures thereof in a gelled form, were designed for use as hand antiseptics in medical and food-handling situations. Their use increased slowly but steadily until 1997, when they were launched into the consumer retail setting in the United States and Canada. Currently (i.e.,1999), use of ethanol-based hand sanitizers is growing in both the professional and consumer arenas worldwide. A few isopropanol-based products (sprays and hand dips) are used in the food industry.

Alcohols have been used as hard-surface (nonskin) disinfectants. Whereas the lack of sporicidal effect limits sterilization applications for alcohols, their general antimicrobial properties can be useful against surface-borne bacteria and other microorganisms. Seventy-percent isopropanol is used as a surface spray in some food-handling situations. A variety of available surface-disinfectant sprays claim effectiveness against bacteria, yeast, fungi, and some viruses. Given the volatility and time-exposure requirements of alcohol, some of these claims are questionable.

Although alcohols are now nearly universally recognized as effective antimicrobial agents, the history of alcohol use for antisepsis, disinfection, and sterilization is replete with controversy in light of seemingly contradictory findings. Issues such as spectrum of activity, concentration levels, time of action, and formulations have been routinely argued in the literature, and practices have varied widely. These differences most always can be traced to methodology and situational factors. It is critically important to specify experimental conditions in detail when considering the antimicrobial aspects of alcohols.

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As a group, the alcohols possess many features desirable for a disinfectant or antiseptic. They have a pronounced bactericidal as well as some bacteriostatic action against vegetative forms, the specific effect being concentration and condition dependent. Alcohols exhibit a wide range of antiviral, antifungal, and antimycosal effects. The low–molecular weight alcohols evaporate readily. They are relatively inexpensive, usually easily obtainable, and relatively nontoxic with topical application. Many alcohols have a cleansing action that is due to their lipid solvency and low surface tension. They are colorless but can be easily colored if needed. Like most chemical disinfectants, their destructive action against spore forms is much less than that against vegetative forms. Alcohols differ from many disinfectants by their lack of residual (persistent) effect. Although studied extensively (Beck, 1984, 1990; Kamm, 1921; Sykes, 1958) as a group, the mode of action of alcohols is not entirely understood. The greatest amount of work has been done with ethanol, followed by the propanols.

PHYSICAL CHEMICAL PROPERTIES

As the chain length of alcohol increases, lipophilicity increases while solubility in water decreases; however, branching the carbon chain length in alcohol improves the solubility of the molecule. The hydrophilic and lipophilic characteristics of alcohols are also expressed as partition coefficient P in systems such as octanol/water or diethyl ether/water. These values are summarized for various alcohols in Table 12.1. Alcohols at higher chain lengths are solids at room temperature, which limits their applications as antimicrobial agents. The short-chain alcohols are inflammable liquids. The flash points for methanol, ethanol, propanols, and tert butanol are lower than 15°C. Therefore, caution should be exercised in handling these alcohols. Safety data for a range of alcohols are presented in Table 12.2.

The concentrations of alcohols are generally given in percentage by weight (g/g or wt/wt) or percentage in volume (mL/mL or vol/vol). Table 12.3 illustrates the per-

TABLE 12.1. *Physical properties of alcohols*

Alcohol	Mol wt	Specific gravity at 20°C	Boiling point (°C)	Melting point (°C)	Solubility in water at 20°C (g/100 g water)	Partition coefficient (log P)[a]	Viscosity (mPa · s)[b]	(°C)[b]
Methanol	32.04	0.791	64.7	−98	Unlimited		0.52	20
Ethanol	46.07	0.789	78.4	−117	Unlimited	−0.58	1.22	20
Propan-1-ol[c]	60.10	0.805	97.2	−127	Unlimited	0.28	2.75	20
Propan-2-ol[d]		0.785	82.0	−89	Unlimited	−0.19	2.27	20
Butan-1-ol[e]	74.12	0.810	117.0	−89	7.7	0.89	2.95	20
Butan-2-ol[f]		0.808	99.8	−114	12.5	0.84	4.21	20
2-Methylpropan-1-ol[g]		0.802	108	−108	9.5	0.65	6.68	20
2-Methylpropan-2-ol[h]		0.779	82.9	24	Unlimited	0.34	3.30	30
Pentan-1-ol[i]	88.15	0.815	138	−79	2.7		3.68	20
Pentan-2-ol[j]		0.808	119	−50	13.5			
3-Methylbutan-1-ol[k]		0.816	131	−117	2.5			
2-Methylbutan-1-ol[l]		0.816	128	70	3.6		5.10	20
2-Methylbutan-2-ol[m]		0.800	102	−12	17.7 (30°C)		3.70	25
Hexan-1-ol	102.20	0.814	157	−45	0.6		0.45	24
Heptan-1-ol	116.21	0.824	175	−34	Slight			
Octan-1-ol	130.23	0.827	195	−16	Slight		8.40	20
Benzyl alcohol	108.13	1.042	204.7	−15.3	3.5			

[a]System: Diethylether/water.
[b]Centipoise.
[c]*n*-Propanol.
[d]Isopropyl alcohol.
[e]*n*-Butanol.
[f]*sec*-Butanol.
[g]Isobutanol.
[h]*tert*-Butanol.
[i]*n*-Amyl alcohol.
[j]*sec*-Amyl alcohol.
[k]Isoamyl alcohol.
[l]*act*-Amyl alcohol.
[m]*tert*-Amyl alcohol.

TABLE 12.2. *Safety data of alcohols used for antiseptic purposes*

Alcohol	Vapor Pressure at 20°C hPa	Vapor density at 20°C	Flash point[a] (°C)	Explosion limits in air (vol%)	Autoignition temp (°C)	Odor threshold (ppm)
Methanol	128	1.1	11	6.0–36.0	385	5
Ethanol	59	1.6	13	3.3–19.0	363	10
Propan-1-ol	19	2.1	15	2.2–13.7	410	30
Propan-2-ol	43	2.1	12	2.0–12.7	399	40
Butan-1-ol	7	2.5	30	1.4–11.3	343	10
Butan-2-ol	17	2.6	23	1.7–9.8	405	0.6
Isobutanol	12	2.6	29	1.7–10.9	430	40
tert-Butanol	40	2.6	11	2.3–8.0	478	73
Pentan-1-ol	3	3.0	48	1.3–10.5	300	1
Pentan-2-ol	3	3.0	41	1.2–9.0	347	70
akt-Amyl alcohol	51[b]	?	50	1.9–9.3	340	
tert-Amyl alcohol	13	3.0	20	1.3–9.6	425	2.3
Hexan-1-ol	1	3.5	63	2.1–7.7	292	5.2
Heptan-1-ol	0.5	4.0	70		350	0.5
Octan-1-ol	0.3	4.5	81	0.8	270	
Benzyl alcohol	0.1	3.7	101		436	

[a]For concentrated solutions.
[b]At 50°C.
Data from Hommel G, *Handbuch der Gefährlichen Güter*, 4th ed. Berlin: Springer, 1980, and Threon JF, *Alcohols, Industrial Hygiene and Toxicology*, Patty FA, ed., 2nd ed., vol 2: *Toxicology*, Fassett DW, Irish DD, eds. New York: John Wiley & Sons, 1963.

TABLE 12.3. *Concentrations as percentages by weight and volume for aqueous ethanol, propan-2-ol and propan-1-ol*

	Concentrations by: Volume at 20°C (% mL/mL)		
Weight (% g/g) Alcohol	Ethanol	Propan-2-ol	Propan-1-ol
40	47.4	47.0	46.5
50	57.8	57.0	56.5
60	67.7	67.2	66.2
70	76.9	76.5	76.1
80	85.4	85.0	84.5
90	93.2	93.8	93.5
95	96.7	97.5	97.2
100	100.0	100.0	100.0
Volume at 20°C (% mL/mL)		Weight (% g/g)	
40	33.4	33.5	34.0
50	42.6	43.0	43.4
60	52.1	53.7	53.9
70	62.6	62.8	63.5
80	73.4	73.5	74.5
90	85.8	86.0	86.0
95	92.5	92.8	93.0
100	100.0	100.0	100.0

centages by volume and weight for ethanol, isopropanol, and propan-1-ol. Because of the volume contraction on mixing, the best way to avoid difficulties in manufacturing of alcohol solutions is to work with compositions by weight rather than by volume.

MODE OF ACTION

Like many chemical disinfectants, alcohols are generally considered to be nonspecific antimicrobials because of a multiplicity of toxic effect mechanisms. This has important implications for the spectrum, speed, and, ultimately, overall effectiveness of alcohols as disinfectants. The predominant mode of action appears to stem from protein coagulation/denaturation (Kamm, 1921). Associated disruptions of cytoplasmic integrity, cell lysis, and interference with cellular metabolism have been reported.

Protein coagulation occurs within concentration limits around an optimum alcohol level. In the absence of water, proteins are not denatured as readily as when water is present. This affords an explanation for why absolute ethanol, a dehydrating agent, is less bactericidal than mixtures of alcohol and water.

Alcohol-induced coagulation of proteins occurs at the cell wall, the cytoplasmic membrane and among the various plasma proteins. It reportedly does not occur in the nucleoproteins (Sobernheim, 1943). Coagulation of plasma proteins is readily detectable by electron microscopy. Coagulation of enzymatic proteins leads to loss of cellular functions. Sykes (1939) determined the

following order for denaturation of *Escherichia coli* bacterial hydrogenases: ethanol > 2-propanol > 1-butanol > 1-pentanol. The interaction of alcohols with proteins raises the issue of interference with antisepsis/disinfection by proteinaceous soils. This topic is discussed in a later section.

Alcohols target the bacterial cell wall, with resultant lysis of the cytoplasmic membrane and release of cellular contents (Pethica, 1958; Isquith and Chesbro, 1963; Craxi et al., 1968). Relatively low concentrations, about twice the minimum inhibitory concentration (MIC), can accomplish lysis. Pulvertaft and Lumb (1948) reported that lysis of microorganisms occurred with many antiseptics at this level, including formalin, mercuric chloride, thimerosal, phenol, and sodium hypochlorite, as well as with the antibiotic penicillin. Lysis was most marked with staphylococci, pneumococci, *Bacillus subtilis,* and *E. coli,* less marked with *Shigella flexneri* and *sonnei,* and only slight with streptococci. The organisms were most susceptible to lysis when the antiseptic was added to the culture during its logarithmic phase, and lysis usually began about the third hour thereafter. The lytic action was thought to be due to using the antiseptic in a concentration that inhibited growth of the microorganisms without inhibiting the autolytic enzymes.

Leece (personal communication, 1954) observed that a suspension of the Campo strain of mycoplasma isolated from humans, estimated to contain 140 μg nitrogen per milliliter, was lysed within a few minutes by tertiary butanol and isopentanols in a concentration of 0.1%. The organisms were not lysed under similar conditions by methanol, ethanol, propanol, isopropanol, and normal butanols. *E. coli*, in a suspension estimated to contain 500 μg dry weight per milliliter, was lysed in the presence of 33% propyl and tertiary butanols. Razin and Argaman (1963) found that *Acholeplasma laidlawii* and mycoplasma were lysed by ethanol in concentrations between 4.3 and 5.4 mol, by propyl alcohol in concentrations greater than 0.84 mol, and by normal butanol in concentrations greater than 0.24 mol.

Antibacterial mechanisms of phenethanol and benzyl alcohol have been studied in some detail. Lucchini et al. (1990) and Corre et al. (1990) found, via electron microscopy, permeabilization of gram-negative (*E. coli* and *Pseudomonas aeruginosa*) cell envelopes and solubilization of the plasmic membrane of gram-positive *Staphylococcus aureus*. Rapid and total leakage of K^+ ions was observed for all bacteria studied. Although a general toxic effect resulting from membrane disruption was demonstrated, the investigators further determined that the lethal effects stopped when protein synthesis was inhibited, suggesting the existence of at least one specific mode of action for the two aromatic alcohols.

Other studies also suggest that alcohols interfere with specific cellular mechanisms. Todrick et al. (1951) noted the concentration-dependent effect of C_3–C_6 alcohols on cholinesterase activity, ranging from inhibition to complete inactivation. Dagley et al. (1950) observed that 0.41*M* ethanol increased the lag phase of *Enterobacter aerogenes*. This effect was decreased by the presence of the amino acids DL-methionine, L-leucine, L-glutamic acid, L-histidine, and DL-tryptophane. L-proline, glycine, DL-alanine, DL-serine, and DL-aspartic acid increased the lag. The researchers concluded that the bacteriostatic action was due to the inhibition of the production of metabolites essential for rapid cell division.

After exposure to various chemical and physical agents, bacterial cells often are judged to be killed when they fail to multiply in suitable growth medium. When incubated in special media, however, these supposedly killed cells frequently can be rendered viable and capable of growth. Heinmets et al. (1954) reported that *E. coli* failed to grow after being exposed to 20% ethanol for 10 minutes, but they grew after being treated with various metabolites. Best results in demonstrating viable cells in the "sterilized" suspensions of treated cells were obtained with cis-aconitic acid, a-ketoglutaric acid, and a mixture of 11 metabolites that included the two already mentioned. A similar "reactivation" effect was observed under conditions of hand disinfection (Freidemann and Stahl, 1969). Likewise, sporadic reports of increased levels of microorganisms on skin following sequential treatment with soap and alcohol may involve such an effect associated with specific test methodologies.

The inhibition of spore germination by ethanol and other alcohols may be due to the inhibition of enzymes necessary for germination. This inhibition is reversible because only the removal of the alcohol from the environment is necessary for germination to take place (Trujillo and Laible, 1970).

STRUCTURE/ACTIVITY RELATIONSHIPS

The physicochemical properties of alcohols are associated with their chemical structure. Factors such as water solubility or miscibility, lipophilicity, solvency, surface tension, vapor pressure, and protein denaturancy vary with chemical structure and help explain corresponding variations in biological activity. Franke and Kramer (1982) provided an extensive review of the general correlation between structure and the biologic effects of alcohols. Other useful summaries include Morton (1983), Freidemann and Truehoff's (1972) work on monovalent alcohols, and the more recent survey by Heeg et al. (1987).

Both alkyl chain length and branching affect antimicrobial activity. The following ranking is generally established and long standing: n-primary > iso-primary > secondary > tertiary alcohols (Rotter, 1996a,b). Wirgin (1904) pointed out that the bactericidal action of the aliphatic alcohols (methyl, ethyl, propyl, butyl, and amyl)

increased with an increase in molecular weight, with the exception of the tertiary alcohols. Testing alcohols in a liquid environment against *Salmonella typhosa,* Tilley and Schaffer (1926) observed an increase in the phenol coefficients from 0.026 for methanol to 21.0 for octyl alcohol. For *S. aureus,* the phenol coefficients varied from 0.03 for methanol to 0.63 for amyl alcohol. Kokko (1939) reported the antibacterial effects of the monovalent aliphatic alcohols increased with boiling point.

Increasing length of the unbranched alkyl chain up to six carbon atoms increases bactericidal effect, whereas further increases in chain length reduce it. Branching of the alkyl group increases water solubility but decreases antimicrobial effect. Of the water miscible alcohols, propan-1-ol is reportedly the most active (Tanner and Wilson, 1943; Rotter, 1984a, 1984b). Tanner and Wilson (1943) tested alcohols containing from 1 to 11 carbon atoms by the agar cup-plate method. Methanol had no noticeable effect on the test organisms. The size of the zones in which no growth of the test organisms took place increased in size from that for ethanol to that for amyl alcohol and then decreased in size for the normal primary alcohol series. The size of the zone of inhibition for primary normal undecyl alcohol, the last alcohol in the series to be tested, was comparable to that for ethanol. In the case of *P. aeruginosa,* growth was stimulated rather than inhibited by the sample of undecyl alcohol. Kubo et al. (1993) evaluated the antibacterial activity of a C_6–C_{20} series of long chain naturally occurring alcohols against *Streptococcus mutans,* observing a correlation between activity and the balance between hydrophilic and hydrophobic portions of the alcohol molecules.

Inactivation of bacterial spores with alcohol also shows a general structure-activity correlation, although data are limited. Morton (1983) found spores of *Bacillus anthracis* to resist methanol completely at concentrations of 0.004% to 95% but to be killed within 48 hours by ethanol at concentrations of 42% to 100% and within 2 to 5 minutes by 30% to 40% isopropanol. Nine months' contact with 100% primary normal methyl, ethyl, propyl, butyl, amyl, hexyl, heptyl, octyl, nonyl, and undecyl alcohols did not kill *B. subtilis* and *Bacillus megatherium* spores (Tanner and Wilson, 1943). Sporicidal activity of alcohols can be enhanced by combination with alkali, mineral acids, hydrogen peroxide, some surfactants, and formaldehyde (Coulthard and Sykes, 1936; Weuffen et al., 1984; Kramer et al., 1987).

REGULATIONS AND GUIDELINES

Antiseptic and disinfectant applications of alcohol are subject to an array of legal and regulatory requirements and are the subject of numerous guidelines. Ethanol, in addition, is subject to religious and societal control based on its intoxication potential. Major considerations for legal and regulatory standards for alcohol include the purpose of use (disinfectant, antiseptic, or sterilant), the type of application (topical or hard surface), and trade factors (importation, registration, taxation). In addition, many countries categorize alcohol-based antimicrobials as being drugs or cosmetics. Each of these factors is a consideration in the development, marketing, and use of alcohol antiseptics and disinfectants.

The United States treats topical alcohol antiseptics as drugs or cosmetics subject to requirements of the Food, Drug and Cosmetic Act (1938) and subsequent amendments. The U.S. Food and Drug Administration (FDA) has regulatory authority for these products; and many, including surgical scrubs, preoperative skin preparations, health care personnel hand washes, and hand sanitizers are covered by regulatory monographs. The latest tentative final monograph for health care antiseptic drug products, proposed rule (*Federal Register,* 1994) recognizes ethanol as safe and effective for these uses and isopropanol as safe and effective for patient preoperative skin preparation. An earlier document provides additional perspective on alcohol as a topical antimicrobial (*Federal Register,* 1982).

Hard-surface disinfectant applications are under the purview of the U.S. Environmental Protection Agency (EPA). Certain food setting uses of both antiseptic and disinfectant products are under the authority of both FDA and the United States Department of Agriculture (USDA), although a shift in the responsibility for disinfectants to EPA exclusively is under way. The U.S. Food Code addresses specific issues of antiseptic and disinfectant use in food service settings. The Bureau of Alcohol, Tobacco and Firearms (BATF) also has regulations for all uses of ethanol, including denatured alcohol.

The U.S. Guidelines covering alcohol antiseptics include those of the Centers For Disease Control and Prevention (Garner, 1996), and the Association of Professionals in Infection Control (APIC; Larson, 1995). Disinfectant uses are addressed in the APIC Guideline for Selection and Use of Disinfectants (Rutala, 1996).

International regulations are similarly complex. Canada treats alcohol antiseptics as drugs and subject to its 1995 monograph. Japan regulates alcohol-based hand sanitizers as soaps, cosmetics, quasidrugs, or medical drugs dependent on formulation and claims. The European Union (EU) has both EU and individual country standards. All EU member states have adopted the Cosmetics Directive 76/768 and EC Directive 65/65 for medicines. National variations, however, still exist in addition to the Directives, resulting in a patchwork of regulatory requirements.

RESISTANCE

Disinfectants and antiseptics have been widely used in health care settings for many decades, serving as a major

component of infection control efforts (Larson, 1996; Rutala, 1995). Routine use of antimicrobials for surface and topical applications also has spread to other professional settings and to the general public. Despite this extensive use, less is known about the mode of action of these biocides than with antibiotic drugs. This has prompted speculation and initial studies on the development of microbial resistance to biocides, especially cross-resistance to antibiotics. Jones (1999) recently reviewed resistance of topical antimicrobials, and McDonnell and Russell (1999) extensively summarized the state of knowledge of microbial resistance to antiseptics and disinfectants.

Resistance is not a significant issue with alcohols, especially at use-level concentrations employed for antisepsis and disinfection. Wille (1976) found no evidence of increase in resistance in 50 passages of *S. aureus* and four gram-negative bacteria in the presence of 3.5% and 7% 2-propanol. A study by Wigert et al. (1979) found the sensitivity of *S. aureus* and some gram-negative bacteria to be decreased using subbacteriostatic concentrations of several alcohols, but the change was immediately reversed with termination of alcohol exposure.

ANTISEPTIC PROPERTIES OF ALCOHOLS

This section presents a summary of antiseptic properties of alcohols, particularly short-chain aliphatic alcohols against vegetative forms of bacteria, bacterial spores, viruses, fungi, yeast, and protozoa will be presented. At the end of this section, the antimicrobial properties of other alcohols, such as phenylethanol, benzyl alcohol, and some long-chain alcohols are described.

Vegetative Bacteria

Alcohol is and has been widely used for the destruction of the vegetative forms of microorganisms preceding such procedures as venipunctures, hypodermic injections, finger pricks, and other procedures that break the intact skin. It is also widely used in some European countries and increasingly in the United States as a surgical hand scrub and as a hand rinse.

During the latter part of the nineteenth century, when ethanol was recommended (Fürbringer, 1888) for disinfection of hands, it was really intended for use as a cleanser rather than a bacteriocide because of its lipid-dissolving properties. Fürbringer (1888) then recommended a mixture of 0.2% sublimate in 3% carboxylic acid as a next step for disinfection. The reduction of bacteria observed after the application of alcohol was explained by the fixation of microbial cells to the skin or by trapping the bacteria in the depth of alcohol-indurated skin surface. This explanation was refuted by other investigations (Wirgin, 1904; Kokko, 1939; Post and Nicoll, 1910), who proved that bacteria were readily killed by alcohols and showed (Bernstein, 1976) that a 2-minute treatment with alcohol did not reduce the release of epithelial cells from the skin.

The antimicrobial activities of various alcohols in terms of their effectiveness either to inhibit growth or inactivate *S. aureus* and *E. coli* are presented in Table 12.4. Higher–chain-length alcohols are more effective than short-chain alcohols and tertiary alcohols are less active than primary or secondary alcohols. Similar observations were made for test organisms on carriers such as batiste patches or glass slide, as shown in Table 12.5. It also can be concluded from these tables that methanol is an effective bacteriocide at concentrations above 50% (Wirgin, 1904; Kokko, 1939; Tanner and Wilson, 1943). Methanol is active on dried bacteria without the presence of water, which is not the case with other alcohols. The addition of methanol at 10% to 96% ethanol for denaturation of protein provides antiseptic efficacy (Lockemann, 1941).

Ethanol is effective as a bacteriostatic agent at 10% (vol/vol). Germination of bacterial spores is inhibited at this concentration (Walthäusser, 1984). Ethanol is bactericidal at concentrations of about 30% and higher, depending on bacterial species, water content, and exposure time (Morton, 1950). Table 12.6 summarizes the bactericidal efficacy of ethanol in suspension tests for various bacterial species. The inactivation time for all the bacteria listed in the table, except *Streptococcus pyogenes*, was 1 minute or less. It is also known (Rotter, 1996b) that a high percentage of ethanol, 90% up to 100%, is less active than lower alcohol concentrations.

Harrington and Walker (1903) tested organisms on moist and dry threads against various concentrations of ethanol. *E. coli* on moist threads was killed after less than 5 minutes' exposure to ethanol at 60% to 99% concentrations, whereas only the concentrations of 50% to 60% killed within 5 minutes when the organisms were dried on threads. Concentrations of 94% and 99% did not kill *E. coli, P. aeruginosa, Staphylococcus epidermidis*, or *S. aureus* in an exposure of 24 hours when the organisms were dried on threads. *P. aeruginosa* on moist threads was killed in 5 minutes by concentrations of 40% to 99%, but on dried threads it was killed only by the concentrations of 40% to 70% in the same period. *S. epidermidis* on moist threads was killed in less than 1 minute by 50% alcohol. Concentrations of 40% to 99% killed in 5 minutes, but when the organisms were dried on threads, only the concentrations of 40% to 70% killed in the same period. Essentially the same results were obtained with *S. aureus*. On moist threads, *S. typhosa* was killed in 5 minutes by concentrations of 30% to 99% but on dried threads only by concentrations of 30% to 80%. *Corynebacterium diphtheriae* on moist threads was killed in 5 minutes by concentrations of 40% to 99% and dried threads by concentrations of 40% to 90%. Thus, these investigators concluded more than three fourths of a cen-

TABLE 12.4. *Comparison of minimum bacteriostatic and microbicidal concentrations of various alcohols in suspension tests*

	Effect: Minimum effective concentrations (% vol/vol)						
	Growth inhib.	Killing effect in suspension test					
	Test organism						
	S. aureus	*S. aureus*			*E. coli*		
Alcohol		1 min	2 min	10 min	1 min	2 min	12 min
Methanol	9	65			60–65		
Ethanol	7	50			45–50		
Propan-1-ol	4	20			17		
Propan-2-ol	4	45			26		
Butan-1-ol	3	9			5		
Isobutanol	3	16			6.5		
sec-Butanol		15			11		
tert-Butanol	5		26			14	
Pentan-1-ol	1			3	2		
Isopentanol			4		2.75		
sec-Pentanol		7.5		4			
tert-Pentanol	3	15			9		
Hexan-1-ol		0.6[a]	5[a]				
Heptan-1-ol		0.12[b]					0.12
Octan-1-ol		0.06[c]			0.06		

[a]In 30 min.
[b]In >30 min.
[c]Converted from % wt/wt.
Adapted from Rotter ML. Alcohols for antisepsis of hands and skin. In: Ascenzi JM, ed. *Handbook of disinfectants and antiseptics.* New York: Marcel Dekker, 1996b:177–233, with permission.

TABLE 12.5. *Comparison of minimum bactericidal concentrations of various alcohols in carrier tests*

	Minimum effective concentrations (% vol/vol) Test organism							
	S. aureus			*E. coli*	*M. tuberculosis*			
Alcohol	2 min	30 min	30 min	0.25–15 s	10[a] min	15 min	30 min	120 min
Methanol		57[a]	70	95			80	90
Ethanol	80	43[a]	60	80	96, –80		80	
Propan-2-ol	50		50	50	60		20	
Propan-1 -ol		23[a]	30	20	50, –50		20	
Butan-1-ol							5	
Isobutanol								10
Propen(l)-ol(3)							20	
Benzyl alcohol						~4		

[a]Dried in sputum on slide.
[b]Converted from % wt/wt.
Adapted from Rotter ML. Alcohols for antisepsis of hands and skin. In: Ascenzi JM, ed. *Handbook of disinfectants and antiseptics.* New York: Marcel Dekker, 1996b:177–233, with permission.

TABLE 12.6. *Bactericidal efficacy of ethanol at various concentrations in suspension tests*

	Killing time (s)		
Bacterial species	60%	70%	80%
Staphyloccus aureus	15	15	10
Staphylococcus epidermidis	30	30	
Streptococcus pyogenes			90
Escherichia coli	60	30	30
Serratia marcescens		10	
Salmonella typhi		10	
Pseudomonas aeruginosa		10	
Mycobacterium tuberculosis	60	30	30

From Walthäußer KH. *Pravis der Sterilization–Desinfektion-Konserierung-Keimidentifizierung-Betriebshygiene,* 3rd ed. Stuttgart: Thieme, 1984.

tury ago that against some common, nonspore–forming bacteria in a moist condition, any strength of ethanol above 40% by volume is effective within 5 minutes, and certain preparations are effective within an exposure time of 1 minute. Against organisms in the dry state, concentrations of 60% to 70% are the most effective, and the same concentrations are equally effective against the organisms in the moist state. Typical results are reproduced in Table 12.7. Thirty-second exposure to 70% ethanol was observed by Hared et al. (1963) to kill microorganisms of ten species when dried on glass surfaces, which indicates that it is possible for an aqueous alcoholic solution to exert its bactericidal action even on dried organisms before it evaporates.

By adding a loopful of suspension of microorganisms to the solution, Post and Nicoll (1910) found that 70% and 50% concentrations of ethanol killed *S. pyogenes, Streptococcus pneumoniae, Neisseria gonorrhoeae,* and *S. typhosa* in less than 1 minute. Even 30% alcohol killed *N. gonorrhoeae* in less than 1 minute.

Morton (1950) tested various concentrations of ethanol against a variety of microorganisms in exposure periods beginning with 10 seconds. To avoid altering the concentrations of the alcohol, the test organisms in 0.5-mL amounts of broth culture were placed in sterile tubes and centrifuged. The supernatant was removed, and the alcoholic solution was thoroughly mixed with the moist cells. One drop of the culture-cell mixture was subcultured at stated intervals. *P. aeruginosa* was killed in 10 seconds by all concentrations of ethanol from 100 to 30% by volume. The lowest concentration of ethanol tested, 20%, killed in 30 minutes. *Serratia marcescens, E. coli,* and *S. typhosa* were killed in 10 seconds by all concentrations of ethanol from 100% to 40% (Table 12.8). Nathanson (personal communication, 1951) stated that 2% ethanol added to sulfonated vegetable oils prevented the growth of *S. marcescens* and *P. aeruginosa* in most cases. Ethanol or isopropanol, in concentrations of 4% to 7%, are used for preservatives of certain disinfectant solutions. The gram-positive vegetative organisms *S. aureus* and *S. pyogenes* were a little more resistant, being killed in 10 seconds by concentrations of 60% to 95%. Exposures of 50 and 90 seconds, respectively, were required to kill the two organisms with absolute alcohol at room temperature. At higher temperatures, the killing action would be expected to be more rapid (Tilley, 1942).

Price (1939) used an original technique in which the ethanol-culture mixture was diluted with water to stop the germicidal action, aliquot portions seeded into plates, and colonies from surviving organisms counted. *E. coli* was killed in less than 60 seconds by 60% ethanol (by weight) and in less than 30 seconds by 80% ethanol. *S. epidermidis* was killed in less than 60 seconds by either 50% or 70% ethanol. *S. aureus* was killed in less than 15 seconds by 70% ethanol. By using essentially the same technique, however, Price (1950a,b) reported that *E. coli* required an exposure of 5 minutes to be killed by 60% ethanol. *S. epidermidis* was not killed in 10 minutes by any concentration of alcohol. Hayes (1949) reported that *E. coli, Proteus vulgaris, P. aeruginosa,* and *S. aureus* were killed in less than 5 minutes when exposed to concentrations of ethanol ranging from 50 to 100%. A concentration of 25% ethanol did not kill these organisms during a 30-minute exposure.

To test the capability of a germicide to penetrate deeper layers of skin, Seelig and Gould (1911) made a pouch of celloidin or living animal tissue at the end of a

TABLE 12.7. *The killing action of various concentrations of alcohol against S. aureus on moist and dried threads*

	Exposure time																	
	Moist threads									Dried threads								
	Minutes					Hours				Minutes					Hours			
Ethanol%	5	10	15	30	45	1	2	7	24	5	10	15	30	45	1	2	7	24
15	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
20	+	+	+	+	+	+	+	+	–	+	+	+	+	+	+	+	+	+
25	+	+	+	+	+	+	+	–	–	+	+	+	+	+	+	+	+	–
30	+	+	+	+	+	+	–	–	–	+	+	+	+	+	+	+	–	–
40	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
50	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
60	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
70	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
75	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
80	–	–	–	–	–	–	–	–	–	+	–	–	–	–	–	–	–	–
85	–	–	–	–	–	–	–	–	–	+	+	–	–	–	–	–	–	–
90	–	–	–	–	–	–	–	–	–	+	+	+	–	–			–	–
94	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	–
99	–	–	–	–	–	–	–	–	–	+	+	+	+	+	+	+	+	+

+, Growth; –, no growth

From Harrington C, Walker H. The germicidal action of alcohol. *Boston Med Surg J* 1903;148:546–522.

TABLE 12.8. *The killing action of various concentrations of alcohol against* S. pyogenes

Alcohol		Exposures of test organisms to germicide																
		Seconds					Minutes											
% by vol	% by wt	10	20	30	40	50	1	1½	2	3	3½	4	5	10	15	30	45	60
100	100	+	+	+	+	+	+	–	–	–	–	–	–	–	–	–		
95	92	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
90	85	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
70	62	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
60	52	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–		
50	42	+	+	–	–	–	–	–	–	–	–	–	–	–	–	–		
40	33	+	+	+	+	+	+	+	+	+	+	–	–	–	–	–		
30	24						+	+	+	+	+	+	+	+	+	+	–	–
25	20						+	+	+	+	+	+	+	+	+	+	+	–
20	16						+	+	+	+	+	+	+	+	+	+	+	–

From Morton HW. Relationship of concentration and germicidal efficacy of ethyl alcohol. *Ann NY Acad Sci* 1950;532:191–196.

glass tube, placed the culture under study inside of the pouch, and immersed it in the disinfectant solution. After varying periods, subcultures were made of the culture within the pouch. *S. typhosa* within celloidin pouches was killed within 6 minutes by 95% and 99.8% ethanol concentrations. Concentrations of 50%, 70%, and 80% did not kill in 1 hour. In a pouch of living rabbit omentum immersed in 95% alcohol, the organisms were alive after 5 minutes but dead after 10 minutes. When placed in a pouch of dried rat intestine and immersed in 99% and 95% ethanols, the organisms were still alive after 18 hours. An important incidental observation was that when the organisms lost their motility they were no longer viable. By making microscopic observations of the organisms inside the pouches, the authors could determine whether the organisms had been killed, and the results of subculturing verified this in every case. In celloidin pouches, *E. coli* was killed in 20 but not in 10 minutes by 95% ethanol, in 60 but not in 30 minutes by 80%, in 18 hours but not in 2 hours by 70%; 50% ethanol did not kill in an exposure of 18 hours. Comparable results were obtained with a staphylococcal culture. When certain germicides were dissolved in alcohol, their germicidal action was increased. An alcoholic solution of iodine killed microorganisms within the pouches quicker than 95% alcohol or aqueous solution of iodine alone.

Alcohol in the body may suppress the normal clearing mechanism of the body for bacteria. The retention of *S. aureus* by the lungs of mice was 3.6 times greater when the mice were given an intraperitoneal injection of ethanol (Laurenzi et al., 1963). When 8% alcohol was supplied in the drinking water and the mice were challenged by intravenous injection of the organisms, the survival time was shorter and the number of staphylococci in the liver greater than in the control group. When the mice were given 5% ethanol in their drink for 3 weeks prior to challenge, the number of staphylococci in the liver was greater, but there was no significant difference between the number of organisms in the spleens and kidneys of the mice in the ethanol and control groups, and the mortality rates were comparable (Wasz-Hockert et al., 1959).

In testing the effect of ethanol against *Mycobacterium tuberculosis,* Smith (1947) observed that 95% alcohol killed the tubercle bacilli in sputum within 15 seconds, absolute and 70% alcohol required 30 seconds, and 50% alcohol required 60 seconds. Tubercle bacilli in water suspensions were killed in 15 seconds by 95% alcohol, in 30 seconds by absolute alcohol, and in 60 seconds by 70% alcohol. When tuberculous sputum was allowed to dry on coverslips and then subjected to the action of the alcohols, 50% alcohol killed the tubercle bacilli in 1 or 2 minutes, depending on the thickness of the smears. In thin smears, 70% alcohol killed the organisms in 1 minute; but in thick smears, 95% alcohol killed the organisms in 20 minutes, and absolute alcohol did not kill in an exposure of 60 minutes. Smith concluded that 95% ethanol was best for wet surfaces, 50% for dry surfaces, and 70% for either wet or dry surfaces. Table 12.9 sum-

TABLE 12.9. *Tuberculocidal effect of ethanol at various conditions*

Condition	Tuberculocidal exposure	
	Conc. (% vol)	Time
In sputum (wet)	100	30 s
	95	15 s
	70	30 s
	50	60 s
In water	100	30 s
	95	15 s
	70	60 s
In sputum (dried)		
Thin layer	70	60 s
	50	60 s
Thick layer	100	>60 min
	95	30 min
	70	>5 min

From Smith CR. Alcohol as a disinfectant against the tubercle bacillus. *Public Health Rep* 1947;62:1285–1295.

marizes the results of Smith (1947), which demonstrated the influence of suspending media, moisture content, and other variables on the tuberculocidal effect of ethanol.

The number of tubercle bacilli has an effect on the killing action of 95% alcohol. When 0.0001 or 0.1 mg of bacilli was used, some subcultures were sterile after the organisms had been exposed for 2 minutes, and all subcultures from the longer exposures were sterile (Cohn, 1934). Of course, inoculum size has an important influence on efficacy of any germicide. Frobisher and Sommermeyer (1953) observed that 80% to 100% concentrations of ethanol were effective in killing tubercle bacilli only if precautions, such as violent shaking, were taken to ensure intimate contact between organisms and alcohol before the sputum became coagulated.

Like most chemical disinfectants, alcohol appears to be more toxic for certain tissue cells than for bacterial cells. Welch and Brewer (1942) found absolute alcohol to be 7.5 times more toxic for the phagocytic cells of human and guinea pig blood than for *S. aureus*.

Using a microtiter technique, it was found that various species of *Spiroplasma* (microorganisms lacking walls) varied in their susceptibility to ethanol as well as to other antibacterial agents, such as formalin, glutaraldehyde, and phenol. A strain of *Spiroplasma* of tick origin, designated SMCA (suckling mouse cataract agent), and two strains of honeybee origin were comparable to *S. aureus* and *E. coli* (cell-wall bacteria) in susceptibility to the antibacterial action of alcohol; the five cultures survived in 30% alcohol for as long as 40 minutes. *Spiroplasma citri* was the most susceptible of the strains tested, surviving 20% ethanol for only 5 minutes. Against phenol, one of the honeybee strains was comparable to *S. aureus* and *E. coli* in susceptibility, whereas the remaining strains of *Spiroplasma* were more susceptible (Stanekk et al., 1981).

The propanols (normal-propyl and isopropyl) are the alcohols of the highest molecular weight that are miscible with water in all proportions. Tanner and Wilson (1943) found normal-propyl alcohol to be the strongest bactericide of the water-soluble alcohols. In a review of the literature on isopropanol, Grant (1923) pointed out that it had no noticeable harmful effect on human skin, although it is slightly more toxic than ethanol. Isopropanol vapors, however, may he absorbed through the lungs and produce narcosis. Senz and Goldfarb (1958) stated that isopropanol was twice as toxic as ethanol and produced greater and longer-lasting toxic effects. Toxic reactions have been reported in children who were given alcohol sponge baths to reduce fever (Garrison, 1953; Wise, 1969).

The bactericidal action of isopropanol is slightly greater than that of ethanol. By making counts of surviving organisms after 30 seconds of exposure to varying concentrations of alcohols, Coulthard and Sykes (1936) found isopropanol to be slightly more bactericidal than either ethanol or methanol for *E. coli* and *S. aureus*.

Powell (1945) reported that *S. aureus* was killed by a 1-minute exposure at 20°C to 50%, 60%, 70%, 80%, and 91% isopropanol solutions, but not by 20%, 30%, and 40% solutions. Other tests showed that the same organisms were killed in 5 minutes by 40% and greater concentrations of isopropanol but not by 10%, 20%, or 30% solutions. *E. coli* was killed in 5 minutes at 20°C by 30% and greater concentrations of isopropanol but not by 10% or 20% solutions. Well-spored cultures of *B. subtilis* and *Clostridium novyi* were not killed in 60 minutes at 20°C by any concentration of isopropanol ranging from 20% to 91%. In tests performed some 10 years earlier, Powell stated that the stocks of isopropanol were contaminated with a saprophytic spore-forming organism, which further substantiates its ineffectiveness against bacterial spores. The sporicidal activity of isopropanol can be increased greatly by adding 5% propylene oxide and maintaining a temperature of 30°C (Hart and Ng, 1975). Tainter et al. (1944) reported that *S. aureus* was killed in less than 10 seconds by a 50% aqueous solution of isopropanol. A 90% solution failed to kill the organisms in an exposure of 2 hours, emphasizing the importance of the presence of water for effective bactericidal action, as in the case of ethanol.

Against tubercle bacilli in dried sputum smears, Smith (1947) observed that the bactericidal activity of isopropanol paralleled that of ethanol in the upper and middle ranges of concentrations but surpassed that of ethanol in the range of lower concentrations. Frobisher and Sommermeyer (1953) obtained results with 70% to 50% isopropanol that were comparable to those obtained with 80% to 100% ethanol against tubercle bacilli and other bacilli in sputum when precautions were taken to ensure intimate contact between the bacteria and alcohol before the sputum became coagulated. For a comparison of the ability of isopropanol to disinfect clinical thermometers with that of ethanol, the publications of Gershenfeld et al. (1951) and Sommermeyer and Frobisher (1952) should be consulted.

Butanols and amyl alcohols are of little importance in this context. Except for the tertiary alcohols, their antimicrobial activity seems higher than that of propanols. Low solubility in water and offensive odor are major reasons for their lack of practical use.

Bacterial Spores

Several investigators (Heuzenroeder and Johnson, 1958; Hared et al., 1963) reported that ethanol has little or no effect against bacterial spores. It is inaccurate, however, to state that alcohols are not sporicidal. Even though spores of *B. subtilis* stay alive in 95% ethanol for years (Walthäußer, 1984), this is not the case for all bacterial spores and for all conditions. This was demonstrated by Regamy (1939) where the spores of anaerobic bacteria survived 10 months in 10% ethanol; at higher ethanol

concentrations of 40% to 80%, the survival rate was reduced to 4 weeks. Morton (1983) also showed that spores of *B. anthacis* were sensitive to ethanol concentrations between 42% and 100% and were killed within 48 hours. With isopropanol, the concentration needed was much lower, that is, 30% to 40% within 2 to 5 minutes; however, methanol was not effective in concentrations of 0.004% to 95% (Kolb et al., 1952). Spores of other species (i.e., *B. subtilis* and *C. novyi*) remained unaffected by exposure of isopropanol over 1 hour at concentrations of 20% to 91% (Powell, 1945).

The sporicidal activity of ethanol, methanol, and isopropanol can be augmented by the addition of agents such as 10% amylmethylcresol, 1% hydrochloric, nitric, phosphoric or sulfuric acid, sodium or potassium hydroxide, hydrogen peroxide, some surfactants, and formaldehyde (Coulthard and Sykes, 1936; Szerémi, 1969; Weuffen et al., 1984; Kramer et al., 1987). Ethanol is reported to have become contaminated with spores from materials immersed in it. Kuhn and Dombrowsky (1932) reported that half of the samples of alcohols tested were contaminated. Fatal infections with clostridium were reported by Nye and Mallory (1923) when ethanol used for surgical instruments was contaminated with bacterial spores. Saegesser (1941) concluded that ethanol was not an adequate method for disinfection when he reported presence of gas gangrene bacilli in 70% ethanol in which syringes were stored.

Viruses

There is no general agreement in the literature regarding antiviral activity of alcohols. It is well established (Luthi, 1954; Rotter, 1996b)) that lipophilic, enveloped viruses are easier to inactivate by alcohols and other general disinfectants than are "naked" viruses. Enveloped viruses extensively studied include vaccinia virus, togavirus, influenza A virus, and rabies virus. Naked viruses also have been investigated quite extensively, including picornavirus, poliovirus, coxsackievirus, and echovirus. Enteroviruses, such as hepatitis A and B, and rotaviruses have been studied for their resistance to chemical and physical influences. A summary of in vitro efficacy of short-chain alcohol against viruses is presented in Table 12.10.

TABLE 12.10. *Inactivation of enveloped viruses by alcohols* in vitro

		Minimum effective concentration Exposure times							
		(min)						(h)	
Virus	Alcohol[a]	0.5	2	3	10	15	30	1	24
Vaccina	M					80			
Dried	E						70	50 60	20
Suspension			70					50	
				80					
					70				
					40				
	P-1					30	15		
		40							
				60					
	P-2				48.5				
					30				
Yellow fever	E						17		
Eastern equine	E							50	
encephalitis	P-2							49.5	
Influenza	E		70						
					70				
					30				
	P-1		40						
	P-2				48.5				
					30				
Mumps	E						36		
Newcastle disease	E		70						
	P-2							25	
Herpes simplex	E				30				
	P-2				20				

[a]M, methanol; P-1, propan-1-ol; P-2, propan-2-ol; E, ethanol.

Adapted from Rotter ML. Alcohols for antisepsis of hands and skin. In: Ascenzi JM, ed. *Handbook of disinfectants and antiseptics.* New York: Marcel Dekker, 1996b:177–233, with permission.

Gordon (1925) reported that enveloped viruses such as vaccinia virus were inactivated by methanol at concentrations of 80% in 15 minutes (Kuwert and Thraenhardt, 1977), 50% in 1 hour, and 20% in 24 hours. As shown in Table 12.10, ethanol inactivated the virus in 1 hour but at concentrations of 50% to 60% even under dried conditions. In a suspension of chick embryo material, inactivation of the virus was attained at 2, 3, and 10 minutes at the respective concentrations of 70% (Bingel and Hermann, 1966; Kewitsch and Weuffen, 1970), 80% (Grossgebauer, 1967), and 70% or 40% (Groupe et al., 1955; Klein and Deforest, 1963). Propanols seem to be more effective against this virus compared with ethanol because inactivations were obtained at lower concentrations within similar or shorter times (Kuwert and Thraenhardt, 1977; Kewitsch and Weuffen, 1970; Grossgebauer, 1967; Groupe et al., 1955; Klein and Deforest, 1963).

Togaviruses, such as those causing eastern equine encephalitis or yellow fever, are inactivated at lower ethanol concentrations of 17% and 50% in 30 to 60 minutes (Frobisher, 1930; Bucca, 1956). Isopropanol has activity similar to that of ethanol. Other disinfectants, such as bichloride of mercury, phenol, and hexylresorcinol and sodium oleate, did not inactivate the virus of yellow fever at useful concentrations (Bucca, 1956). Against the virus of mouse encephalomyelities, ethanol at 20% demonstrated a loss of activity exceeding 95% after 45 minutes of exposure.

Kewitsch and Weuffen (1970) found that, at an optimal concentration of 70% ethanol, inactivation of influenza A occurred in 2 minutes. The same results were reported with propan-1-ol at 40%, and isopropanol caused inactivation in 10 minutes at concentrations of 30% and 48.5%, respectively (Klein and Deforest, 1963).

Ethanol was effective at 36% concentration against mumps within 30 minutes (Brahms et al., 1955), and Newcastle disease virus was inactivated by 70% ethanol in 3 minutes (Cunningham, 1948) or by 25% propan-2-ol in 1 hour (Tilley and Anderson, 1947). Herpes simplex virus was killed by 30% ethanol or 20% isopropanol within 10 minutes (Klein and Deforest, 1963).

It can be concluded that for lipophilic, enveloped viruses, alcohols are quite effective when used at appropriate concentrations. These viruses also follow the same order of susceptibilility against bacteria: methanol < ethanol < isopropanol < propan-1-ol.

Klein and Deforest (1963) studied the virucidal effect of seven general types of viruses against ethanol and isopropanol. Although adenoviruses do not number among enveloped viruses, they behave like them in their tenacity. Fifty percent ethanol or isopropanol was required to kill this virus in 10 minutes in a suspension test model.

Compared with lipophilic enveloped viruses, naked viruses are more difficult to inactivate with higher alcohols than with ethanol. Table 12.11 summarizes in vitro inactivation of picornavirus by various aliphatic alcohols. Seventy percent ethanol was effective within 2 minutes against poliovirus type A (Kewitsch and Weuffen, 1970). Klein and Deforest (1963) showed that 70% ethanol in a suspension test also was effective within 10 minutes.

TABLE 12.11. *Inactivation of picornaviruses alcohols in vitro*

		Minimum effective conc. (%)							
		Exposure times (min)							days (d)
Virus	Alcohol	1	2	4	6	8	10	240	6
Poliovirus type 1	E		70						
							70		
								25	
	P-2						95		
	CP				100				
Coxsackie B	E						60		
	P-2						95		
	CP		100						
Echo 6	E						50		
	P-2						90		
Echo 9 (Hill)	Cp					100			
Echo 11	M	76							
	E	76[a]							
Hoof-and-mouth disease	E								25
Hepatitis A	E	70[a]							
	P-2	45[a]							

[a]Not effective at this concentration.

E, ethanol; P-2, propan-2-ol; CP, commercial preparation of ~90% ethanol, 10% propan-2-ol, and 0.1% tetrabromo-6-methylphenol.

Adapted from Rotter ML. Alcohols for antisepsis of hands and skin. In: Ascenzi JM, ed. *Handbook of disinfectants and antiseptics.* New York: Marcel Dekker, 1996b:177–233, with permission.

TABLE 12.12. *Activity of short alcohols against enteric viruses*

Alcohol	Minimum effective conc. (%)[a] Virus tested		
	Rota	Astro	Echo 11
Methanol	>50	90	76
Ethanol		90	76[b]
Propan-1-ol	40	No effect	No effect
Propan-2-ol	40	No effect	No effect
Butan-2-ol	30	No effect	No effect

[a]Minimum concentration reducing virus titer by at least 4 $\log^{10}$ in 1 min in a suspension test system.

[b]Only 3-log reduction.

From Kurtz JB, Lee TW, Parsons AJ. The action of alcohols on rotavirus, astovirus and enterovirus. *J Hosp Infect* 1980;1:321.

Poliovirus type A was also studied by Steinmann et al. (1990) and Tyler et al. (1990) using ethanol and isopropanol. On the other hand, extensively tested antiviral activities of a commercial preparation containing 78.2% (wt/wt) of 95.3% (vol/vol) ethanol [about 90% vol/vol, 10% (vol/vol)] isopropanol and 0.1% tetrabromo-6-methylphenol in a suspension test and demonstrated a 4-log reduction in 6 minutes for viruses of polio type 1 but only a 2 log reduction in polio type 2 in 10 minutes. The titer of coxsackievirus B4 was reduced 4 logs in 2 minutes; but with coxsackievirus B3, this reduction was achieved in 10 min (Schurmann and Eggers, 1983). A strain of echovirus 9 was reduced 4 logs in 8 minutes by this commercial product. These researchers, however, mentioned considerable differences between the five strains tested. A strain of echovirus 11 was sensitive to methanol at 76% concentration in 1 minute but not to other alcohols such as ethanol, propanols, or butanols, even at 90% (Kurtz et al., 1980).

Hepatitis A is known for its resistance to chemical and physical agents. In a recent study (Rotter, 1996b), as many as 20 disinfectants were tested for their capability of inactivating, within 1 minute, the virus contained in a fecal suspension and dried on polished stainless steel disks. Only preparations with 2% glutaraldehyde, sodium hypochlorite, and a quaternary ammonium compound in 23% HCl were effective. Ethanol at 70% and isopropanol at 45% were ineffective.

Alcohols are effective against rotaviruses and other enveloped enteric viruses in the same order from methanol to butan-2-ol (Tables 12.12 and 12.13). Even though animal rotaviruses have been shown to be relatively resistant to a number of common disinfectants and antiseptics, Sattar et al. (1983) reported that a formulation of 70% isopropanol and 0.1% hexachlorophene produced a 3-log reduction after 1-minute exposure against human rotavirus. In contrast, astroviruses are sensitive to high concentrations: 90% methanol and ethanol (Kurtz et al., 1980).

Human immunodeficiency virus (HIV) is a retrovirus that is readily inactivated by most chemical disinfectants, including alcohols. Using reverse transcriptase as an indicator, Spire et al. (1984) reported a 99% reduction in activity after 5 minutes of exposure with 19% ethanol and complete inactivation of HIV with concentrations greater than 20%. They recommend 25% ethanol or 1% glutaraldehyde as effective means for disinfecting instruments contaminated with HIV. Martin et al. (1985) showed inactivity of HIV in 50% ethanol, 35% propan-2-ol within 2 to 10 minutes.

The hepatitis B virus was considered extremely resistant to chemical disinfectants. Bond (1983) and Kobayashi et al. (1984), however, found that 70% isopropanol and 80% ethanol inactivated this virus in 10 minutes and 2 minutes, respectively.

Fungus

The effectiveness of short-chain alcohols mainly ethanol against fungus and yeast have been well documented in the literature: Wallhäusser (1984), Szerémi (1969), Koch and Koch (1969), Kruse et al. (1963, 1964), Emmons (1933), Loewenthal (1961), and Gildemeister et al. (1930). Table 12.14 summarizes the fungicidal effect of ethanol against various fungal species. As seen from

TABLE 12.13. *The virucidal action of ethanol and isopropanols on seven viruses*

Virus	Lowest concentration inactivating in 10 min		Lipid envelope	Lipophilic
	Ethyl	Isopropyl		
Poliovirus, type 1	70%	95% (Negative)		
Coxsackie B-1	60%	95% (Negative)		
ECHO 6	50%	90%		
Adenovirus, type 2	50%	50%		+
Herpes simplex	30%	20%	+	+
Vaccinia	40%	30%	+	+
Influenza, Asian	30%	30%	+	+

From Klein M, Deforest A. Antiviral action of germicides. *Soap Chem Spec* 1963;39: 70–72, 95–97.

TABLE 12.14. *Effect of ethanol against various fungal species*

Fungus	Minimum effective conc. (%) Exposure time (min)				
	1	5	30	60	Not stated
Yeasts		75			
Candida albicans	35				
C. krusei	35				70
Cryptococcus neoformans					70
Histoplasma capsulatum[a]					70
Blastomyces dermatifidis[a]					70
Coccidioides immids[a]		50			
Dermatophytes			80		
Microsporum gypseum (spores)			85	70	
M. audouinfi (on naturally infected hairs)					70
Aspergillus niger	35				90
Penicifflum tardurn (conidia)					(70–96)

[a]Tissue and culture phase.

Adapted from Rotter ML. Alcohols for antisepsis of hands and skin. In: Ascenzi JM, ed. *Handbook of disinfectants and antiseptics.* New York: Marcel Dekker, 1996b:177–233, with permission.

the table, a 35% concentration of ethanol is required to kill *Aspergillus niger* within 1 minute. A higher concentration of methanol and a lower concentration of propanols are required for the same effectiveness.

Protozoans

The activity of alcohols against protozoa is not well studied. In a suspension test, tropbrozoits of *Toxoplasma gondii* were inactivated in a 30% ethanol solution and 20% propan-1-ol solution within 3 minutes (Wachtel et al. 1969). The oocysts of *T. gondii* were killed in 1 hour in 95% ethanol solution and within 7 days in 20% ethanol solution (Duby et al., 1970a, 1970b) This information has limited importance for laboratory workers handling these stages of protozoans of toxoplasma. Little is known about the effectiveness of alcohols against persistent stages of protozoa.

Miscellaneous

The antimicrobial properties of other alcohols such as phenyl ethanol, benzyl alcohol, and other long-chain alcohol is briefly described here. Phenylethanol (benzylcarbinol) was discovered by Lilley and Brewer (1953) to have a unique property among the alcohols in that it has a greater inhibitory effect against gram-negative than against gram-positive organisms. By incorporating 0.25% phenylethanol in the culture medium, it is possible to isolate gram-positive organisms from material containing troublesome contaminating gram-negative organisms such as *Proteus* species.

Mycoplasmas have been observed to be totally inactivated within 20 minutes by exposure to 1% phenylethanol, but enveloped viruses resisted such treatment (Staal and Rowe, 1974). The stable L-form of *Streptococcus faecium,* but not the parent *S. faecium,* was lysed by 0.5 to 0.6% (vol/vol) phenylethanol (King, 1974).

Corre et al. (1990) investigated the lethal effects of phenylethanol by determining its bacteriostatic and bactericidal activities using electron microscopy, K+ leakage measurements, and lysis of spheroplasts *(E. coli* and *P. aeruginosa)* and protoplasts *(Entercoccus faecium)* and were able to explain the lethal effects of phenylethanol. Their results showed that phenylethanol has broad-spectrum bactericidal properties. The lethal activities (reduction of 5-log viability) for the strains used was obtained in a short time (5 minutes) and in a narrow range of concentrations (100–200 mM). Their results pointed to a rapid death of treated cells and showed evident alterations of the cell structures. These alterations seriously disturb the cellular physiology and play an important role in the mechanism of action of phenylethanol.

Of the aromatic alcohols, benzyl alcohol appears to be the only one tested in recent years. It is soluble in water with difficulty, which perhaps explains its limited use. Prombo and Tilden (1950) found 4% benzyl alcohol by weight to be less effective than 70% ethanol (either by weight or by volume) or isopropanol (99%) in preventing infection in mice with *S. pneumoniae.* Benzyl alcohol, 4% by weight, was slightly better than ethanol (70% by weight) or isopropanol (99%) in protecting mice against infection with *S. pyogenes.*

The fate of *P. aeruginosa* was determined in saline alone, 0.9%, and in saline, 0.9%, containing 0.9% benzyl alcohol; 1 mL of a 1:100 dilution of an overnight culture of *P. aeruginosa* in broth was added to 150-mL bottles of

saline and saline plus 0.9% benzyl alcohol. The contents of the bottles were thoroughly mixed by shaking, and the bacterial concentrations were determined immediately and at 24-hour intervals by making pour-plate preparations of appropriate dilutions of the contents of each bottle. It cannot be overemphasized that "bacteriostatic" preparations are not necessarily sterile preparations.

Dichlorobenzyl alcohol has broad-spectrum activity as an antimicrobial agent and is used in cosmetics and pharmaceuticals. Ostergaard (1994) evaluated the antimicrobial effects of this alcohol and sodium benzoate against 115 strains of dental plaque. The MIC for dichlorobenzyl alcohol to the reference strain of *Actinobacillus actinomycetermcomitans* was 723 μM and to *P. gingivatis* and *Candida albicans* was 1,446 μM. Generally, all microorganisms studied were 20 to 30 times more susceptible to dichlorobenzyl alcohol than to sodium benzoate.

Lucchini et al. (1990) investigated the antibacterial properties of phenolic compounds and aromatic alcohols using growth inhibition, lethal effect, and cytological damage. They determined the role of protein and RNA synthesis in the bacterial action. Antimicrobial agents studied by them included benzyl alcohol, phenylethanol, phenoxyethanol, phenol, thymol, carvacol, and resorcinol. This study showed that thymol and carvacol were most effective against *S. aureus, E. faecium,* and *E. coli* at concentrations of 5 mM and *P. aeruginosa* at 10.5 mM. Phenylethanol and phenol were almost equal in their activity, whereas phenoxyethanol, benzyl alcohol, and resorcinol were weaker. The activities of these alcohols were linked to physicochemical properties as supported by membrane alteration of gram-negative bacteria; lipophilia varies inversely with antibacterial activity. These researchers studied role of protein and RNA synthesis in bactericidal activity by adding chlortetracycline and chloramphenicol. The inhibition of protein synthesis led to the suppression of bactericidal effects of phenyl ethanol and benzyl alcohol against *S. aureus*.

Maximum inhibition of growth of *Mycoplasma gallisepticum* and *M. pneumoniae* was obtained by the saturated primary alcohols varying in chain length from 16 to 19 carbon atoms at 64 ppm concentration. The unsaturated alcohols oleyl, linoleyl, and linolenyl were less effective as inhibitors. The inhibitory effect of 48 ppm concentrations of stearyl alcohol was not significantly mitigated by a concentration of 128 μm cholesterol (Fletcher et al., 1981).

Kubo et al. (1993) studied the antibacterial activity of naturally occurring long-chain alcohols against *S. mutans,* a primary bacterium associated with dental caries. Maximum activity was found to depend on the hydrophobic chain length. Alcohols from C_6 to C_{20} chain length were included in the study. The MIC values ranged from 6.25 to 800 μg/mL against *S. mutans*. Optimum activity was found in 1-dodecanol and 1-tridecanol. The activity dropped off more rapidly above C_{13} than below C_{13}. They found an apparent correlation between antibacterial activity against *S. mutans* and the carbon chain length of the alcohol. These results generally were applicable to naturally occurring isoprene long-chain alcohols. For example, a sesquiterpene alcohol (farmorol) was active against *S. mutans* since its chain length was comprised of 12 carbon atoms, while a similar sesquiterpene alcohol exhibited less activity because its chain length was 10 carbon atoms.

ALCOHOLS FOR CHEMICAL DISINFECTION ON HARD SURFACES

Chemical germicides classified as disinfectants are, by definition, products specifically used to inactivate microorganisms on inanimate objects. Especially in health care environments, they are commonly classified as high-, intermediate-, or low-level disinfectants and labeled as such for all proprietary formulations registered by the EPA. The type of disinfectant chosen depends on the item being disinfected and its level of risk in transmitting infection. Spaulding (1964) reviewed the value of alcohol as a surgical germicide. Both ethanol and isopropanol are rapidly bactericidal intermediate-level disinfectants that are remarkably active against the tubercle bacillus (Spaulding, 1972). Both ethanol and isopropanol are effective against all types of vegetative bacteria and fungi, but neither is sporicidal (Prince, 1983). The spectrum of activity of alcohols against viruses varies with the type of alcohol (Bellamy, 1995). Ethanol is broadly viricidal, but the activity of isopropanol is limited primarily to lipid-containing viruses. Alcohols at concentrations of 70% are a reasonable choice for intermediate-level disinfection for noncritical and certain types of semicritical instruments that can be submerged for 10 min. Because alcohols are poor cleaners and evaporate rapidly, they are not appropriate for use on environmental surfaces.

Spaulding (1939) observed that 70% ethanol killed *Candida albicans, E. coli, S. pyogenes,* and *P. aeruginosa* on knife blades contaminated with pus or blood within 1 minute; rarely was exposure of 2 minutes required. *S. aureus* was slightly more resistant when the pus and blood were dried. Exposures of 10 minutes were required to kill the organisms in dried pus and 5 minutes in dried blood. *Clostridium tetani, Clostridium perfringens,* and *B. anthracis* were not killed after an exposure of 18 hours, except *B. anthracis* in wet pus, which was killed in that period. Although Ziegler and Jacoby (1956) reported that soaking in 70% ethanol completely destroyed vegetative bacteria on airways, endotracheal tubes, and cuffs within 15 minutes, Winge-Heden (1962) reported that such soaking did not always sterilize rubber tubing. Force and Kerr (1920) reported that an exposure of 4 minutes to 50% ethanol adequately disinfected oral glass thermometers if they were wiped with a cotton sponge wet with water to free them of mucus before placing them in the alcohol.

Sommermeyer and Frobisher (1952) contaminated glass rods or unfilled oral thermometers with a thin film of tuberculous sputum that was strongly positive for acid-fast bacilli by direct smear examination. Cultures of *Corynebacterium diphtheriae* were added to the samples of sputum, which contained staphylococci, streptococci, and other bacteria in addition to the acid-fast bacilli. The contaminated thermometers were allowed to dry for 30 minutes. Because this level of contamination probably exceeded that encountered in ordinary practice, the results erred on the side of safety. These researchers found that wiping the thermometers with cotton and a mixture of equal parts of 95% ethanol and tincture of green soap before placing in the disinfectant decreased the number of viable organisms in practically every case. After the cleaning procedure, immersion of the thermometers for 10 minutes in 70% ethanol or isopropanol containing 0.5 or 1% iodine reduced the number of viable organisms to a low number. Aqueous iodine solutions or 70% solutions of ethanol or isopropanol were nearly as effective as the alcoholic iodine solutions. The importance of wiping the thermometers was emphasized by Ryan and Miller (1932).

Gershenfeld et al. (1951) tested various agents for their ability to disinfect 2-inch segments of thermometers contaminated with bacterial cultures. Ethanol in 95%, 70%, and 50% concentrations of isopropanol in 70% and 50% concentrations and 2% iodine, aqueous solution and tincture, were tested. *S. pneumoniae* was killed by all disinfectants within 20 seconds, and *S. pyogenes* was killed by all disinfectants within 20 seconds, except 95% ethanol, which required 60 seconds. Cultures of these two organisms were used to contaminate the thermometer segments and, without drying, the segments were transferred to the disinfectants. This may be one factor that accounts for the fast killing action in the experiments. Ecker and Smith (1937) reported that thermometers contaminated with sputum from cases of lobar pneumonia were not sterilized by exposures for as long as 30 minutes to 70% and 95% solutions of ethanol. This is difficult to interpret in light of the findings of Sommermeyer and Frobisher (1952) and of Gershenfeld et al. (1951) with pure cultures of *S. pneumoniae*. The sputum may have exerted a protective action, as Gershenfeld et al. observed for plasma.

The activity of alcohols in the presence of organic material, particularly blood, pus, or feces, was investigated to a limited extent. Although a number of researchers reported continued activity of the alcohols in the presence of organic material (Spaulding, 1939; Smith, 1947; Springthorpe et al., 1986, Larson and Bobo, 1992), others reported reduced effectiveness. (Gershenfeld et al., 1951). Wallbank (1985) stated that 80% (vol/wt) ethanol was neutralized by rabbit blood in his laboratory, but study methods were not described. More recently, Larson and Bobo (1992) conducted a study to show the effect of blood on the antimicrobial activity of several agents such as chlorhexidine gluconate (CHG), providone–iodine, 70% isopropanol, and 70% ethanol with 0.5% CHG along with nonantimicrobial soap. This study concluded that several of these topical products continue to show antimicrobial properties in presence of blood. This is a particular advantage for the use of alcohol products in emergency situations.

The efficacies of selected disinfectants against *M. tuberculosis* on stainless steel surfaces were investigated with sputum as the organic load (Best et al., 1990). Ethanol (70%) was effective against *M. tuberculosis* only in suspension in the absence of sputum. Van Buren et al. (1994) investigated the efficacy of alcohols against HIV in the presence of high- and low-protein concentrations. High titers of HIV were inactivated by 70% ethanol, independent of the protein load. With virus dried onto a glass surface, the rate of inactivation decreased when high levels of protein were present. Mbithi et al. (1990) studied the chemical disinfection of hepatitis A virus on environmental surfaces. In this study, stainless steel disks contaminated by a fecal suspension of hepatitis A virus were used to evaluate the antimicrobial efficacy of various formulations. Of the 20 formulations tested, only 2% glutaraldehyde, a quarternary ammonium formulation containing 23% hydrochloric acid, and sodium hypochlorite reduced the virus by greater than 99.9%. Products containing phenolics, alcohols, or solutions of acetic, citric, and phosphoric acids were unable to do so.

Maki et al. (1991) investigated the effect of providone–iodine, ethanol, and CHG for the prevention of infection associated with central venous and arterial catheters in a prospective study involving 668 catheters. They concluded that the use of 2% CHG rather than 10% providone–iodine or 70% ethanol for cutaneous disinfection before insertion of intravascular device and for postinsertion site care can reduce the incidence of device-related infection substantially. The ability to disinfect latex gloves between procedures with 70% ethanol was investigated using five common microorganisms (*S. aureus, E. coli, P. aeruginosa, P. vulgaris,* and *Klebsiella* species). Seventy percent ethanol was found to provide a high level of disinfection on porous/nonfissured surfaces and to provide a better level of asepsis than untreated gamma-irridiated gloves (Grinnell, 1998). Best et al. (1994) investigated the use of a combined carrier test for disinfectants using a mixture of five types of microorganisms. They found that, of the 11 products tested, 11% alkaline gluteraldehyde, 0.6% sodium hypochlorite, and a 0.4% quarternary ammonium compound containing 23% hydrochloric acid were the most effective against all five challenge organisms. Seventy percent ethanol alone and providone–iodine were effective against *S. aureus,* the mycobacterium, and the fungus. The activity of disinfectants against poliovirus (Sabin 1an) was assessed using both surface and suspension tests (Tyler et al., 1990). Gluteraldehyde (2%) and high concentrations of

hypochlorites were effective in 1 minute in the surface tests, whereas 70% ethanol showed variable results, and 70% isopropanol was ineffective in 10 minutes. In the suspension tests, 70% ethanol was ineffective in 1 minute.

ALCOHOLS FOR SKIN DEGERMING

It is not possible to sterilize the skin; the best one can hope to accomplish is to reduce the number of viable resident organisms on or in the skin and to destroy the pathogenic organisms that may be on the skin as transients. Price (1939) pointed out that, in the surgical scrub, immersion of the hand and arms in a 65.5% solution of alcohol by weight for 1 minute was as effective as scrubbing for 4.2 minutes in reducing the number of bacteria on the skin. This effectiveness of alcohol has been amply confirmed (Pohle and Stuart, 1940; Pillsbury et al., 1942; Hatfield and Lockwood, 1943; Gardner, 1948; Story, 1952; Rotter, 1984; Ayliffe, 1984; Larson et al., 1986; Rotter, 1996a,b, 1997; Rotter et al., 1998; Paulson et al., 1999; Kampf et al., 1999).

Using a testing procedure similar to that of Price, Hatfield and Lockwood (1943) concluded that ethanol in strengths of 95% or 70% by weight was preferable to any of a group of commercially prepared agents available for skin degerming at that time. The ideal concentration was 95%, but for economy the concentration could be reduced to 70%. Pillsbury et al. (1942) also observed that 80% or more by volume produced a satisfactory decrease in the number of viable bacteria remaining on or in the skin. They expressed doubt about the necessity of alcohol of an exact concentration for clinical use so long as the concentration exceeds 70% by volume.

Skin bacteria have been described as "transients" and "residents" (Price, 1938a,b). The transient bacteria lie free on the skin or are loosely attached by lipoidal substances. Their removal is relatively easy compared with removal of the resident bacteria, which are a relatively stable population in size and kinds. The terms *exposed* and *sheltered* flora, in place of transient and resident flora, were proposed by Evans and Mattern (1980). They observed that in 15 persons with a sparse flora in the antecubital fossa, no surviving bacteria were detected after alcohol treatment (60 seconds); in 10 persons who had a more abundant flora, viable bacteria remained, and their number bore no quantitative correlation with the surface flora. Following scrubbing of the skin after alcohol treatment, sheltered or resident bacteria could be demonstrated in each of the persons studied. Lacey (1968) cleaned the skin on the forearm with alcohol, applied *S. aureus*, and covered it with an occlusive dressing for 5 hours. The degree of decrease in bactericidal activity of the skin was about the same as for soap and was thought to be due to the removal of bactericidal substances from the skin. Lowbury et al. (1979) noted that the bacterial counts on alcohol-scrubbed hands continued to drop for several hours after gloving; they concluded that this was probably a result of the continued death of damaged organisms.

Studies of skin antiseptics generally are divided into those that assess the effects on transient, artificially contaminating flora, and those that assess effects on colonizing or resident flora. Many of these studies vary in design and with regard to important variables, such as contact time and method of product application. Sebben (1983) noted that 70% ethanol destroyed 90% of cutaneous bacteria after 2 minutes of contact but emphasized the necessity of maintaining moist conditions throughout the entire application time.

Effect of Alcohols on Contaminating Flora (Transient Microorganisms)

In 1977, Rotter et al. developed a test method for the evaluation of health care personnel handwash products. The method, with some modifications, has been adopted by the German and Austrian governments for testing health care personnel handwashing products (Deutsche Gessellschaft für Hygiene und Präventivmedizin, 1981). The method includes artificial contamination of the hands with *E. coli* and decontamination with two 30-second applications of 3 mL of 60% (by volume) isopropanol as a standard compared with any test product applied according to manufacturer's directions. These researchers reported (Rotter, 1981; Rotter, 1984a,b; Rotter et al., 1986) 4 or greater $\log_{10}$ reductions with all alcohols tested, in comparison with 2 to 3 $\log_{10}$ reductions with soaps containing phenolic antimicrobials, CHG, or providone–iodine. Efficacy in those studies was greatest for 50% to 60% n-propanol and comparable for 60% isopropanol and 70% to 80% ethanol. Recently, a proposed European standard, *Surgical Hand Disinfectants—Test Method and Requirements,* was released for inquiry by the European Committee for Standardization (European Committee for Standardization, 1997b). This standard is similar to standards regulating testing products for the "hygienic hand rub" (European Committee for Standardization, 1996) and for the "hygienic hand wash" (European Committee for Standardization, 1997a). In a study using the proposed method, 90% isopropanol was as effective as 60% n-propanol, the proposed test reference. The effects of two different contamination and sampling techniques on the results of hygienic handrub and hygenic handwash procedures (Rotter and Koller, 1991; Rotter, 1996a, 1996b) have been examined (Rotter and Koller, 1992). No major differences were found between the techniques.

Ulrich (1982) contaminated hands with *Micrococcus roseus* and used a glove-fluid sampling protocol to test products containing 7.5% providone–iodine and a combination of 70% isopropanol and 0.5% CHG. He reported 2

to 3 $\log_{10}$ reductions after 5, 10, 15, 20, and 25 repetitive contamination and degerming cycles, with the alcohol-based product significantly better at each test point. Aly and Maibach (1980), using a similar contamination/treatment procedure and sampling protocol, reported the same combination of 70% alcohol and 0.5% chlorhexidine to be significantly superior to 70% isopropanol in reducing counts of *S. marcescens*. Marples and Towers (1979) developed a model to assess the transfer of organisms by contact. They contaminated a fabric-covered bottle with *Staphylococcus saprophyticus*. Subjects grasped the contaminated bottle, then a sterile fabric-covered bottle, and the numbers of organisms transferred were counted. Washing hands with plain soap reduced transfer by 95%, whereas washing in 500 mL of 70% ethanol for 30 seconds reduced transfer by 99.9%. Fifteen minutes after testing, the contaminating organism was still present on the hands of subjects who washed with soap, but it was undetectable on alcohol-treated hands. An alcohol-impregnated towelette and a small volume (0.2-mL) of 80% ethanol resulted in reductions of a lesser magnitude, 80% and 93%, respectively.

Casewell et al. (1988) demonstrated complete removal of an epidemic multiply resistant strain of *Kiebsiella* using 0.5% chlorhexidine in isopropanol. Wade et al. (1991) demonstrated that 0.5% chlorhexidine in isopropanol was the most effective agent for the removal and infection control of epidemic vancomycin-resistant *E. faecium* and gentamicin-resistant, multiply resistant strain of *Enterobacter cloacae*. Eckert et al. (1989) demonstrated that handwashing with soap was inadequate and that 60% to 70% isopropanol was required for removal of aerobic gram-negative bacilli, such as Enterobacteriaceae, transiently acquired from patients. Similarly, a bland soap hand wash was ineffective in preventing transfer by hand of gram-negative bacteria to catheters following brief contact with a heavily contaminated patient source; an alcohol hand rinse was generally effective (Ehrenkranz and Alfonso, 1991). Maki et al. (1991) compared the effectiveness of 10% providone–iodine, 70% isopropanol, and 2% aqueous CHG for the prevention of infection associated with central venous and arterial catheters. CHG resulted in the lowest incidence of local catheter-related infection (2.3% versus 7.1% and 9.3% for isopropanol and providone–iodine, respectively) and catheter-related bacteremia (0.5% versus 2.3% and 2.6% for isopropanol and providone–iodine, respectively).

In a study on the effectiveness of hand washing and hand disinfection for the removal of nosocomial pathogens from heavily contaminated hands (Kjelen and Anderson, 992), 0.5% CHG in 70% isopropanol was most effective, followed by 70% ethanol and, to a lesser extent, 40% isopropanol. Comparison of the efficacy of 62% ethanol, 70% isopropanol, and benzylalkonium chloride-based hand sanitizers using the health care personnel hand wash protocol indicated that all three had equivalent efficacy at greater than 2-log reduction after the first wash, whereas the benzylalkonium chloride hand sanitizer demonstrated residual efficacy (Dyer et al., 1998).

Effects of Alcohols on Colonizing Flora (Resident Skin Microorganisms)

In a series of studies conducted by a group of British investigators (Lilly and Lowbury, 1971; Lowbury and Lilly, 1973, 1975; Lowbury et al., 1974; Lilly et al., 1979), plain soap, 4% chlorhexidine in detergent base, 10% providone–iodine detergent, and 70% isopropanol with 0.5% CHG were compared in a surgical scrub protocol. The alcoholic CHG produced the greatest initial reduction in bacterial flora (98% compared with 87% for CHG detergent, 68% for providone–iodine, and 13% for soap), but the CHG and providone–iodine detergents had almost identical effects to the alcoholic product (> 99% reductions in flora) after six applications. Larson et al. (1987) found that regrowth of colonizing flora after 4 hours of gloving was minimal after a 5-minute scrub with a formulation of 70% ethanol and 0.5% CHG. Ayliffe (1984) reported continued reductions in bacterial counts following hand degerming with 70% isopropanol as well as alcoholic CHG and CHG detergent after wearing gloves for 3 hours. Alcoholic preparations are efficacious as surgical scrubs, perhaps in part because of their superior activity in reducing bacterial populations under the fingernails (Gross et al., 1979; McGinley et al., 1988; Larson et al., 1990).

Several investigators evaluated the effectiveness of alcohols as hand rinses after short contact times. Morrison et al. (1986) compared three alcohol-based hand rinses, including 70% isopropanol, 0.5% CHG in 70% isopropanol, and a 60% isopropanol formulation containing evaporative retardant in 14 subjects. The 60% isopropanol with evaporative retardant was associated with significantly greater reductions after each of four consecutive handwashes. Similarly, Larson et al. (1986) reported significant reductions over baseline counts among subjects using this formulation of isopropanol after a single 15-second application. After using this formulation 15 times per day for 5 consecutive days, subjects using either one of two alcoholic hand rinses, 70% isopropanol, or 4% CHG in detergent all had significant reductions in their colonizing flora, but the two alcoholic hand rinses continued to be associated with the greatest reductions. There was no significant change in bacterial counts among subjects using a nonmedicated control soap. In another study, Larson et al. (1987) demonstrated a significant dose response with two alcohol hand rinses: subjects using a 3-mL hand rinse had significantly greater reductions in bacterial flora counts than did subjects using 1-mL rinse. Based on these data, alcohols deserve serious considera-

tion for use as surgical scrubs and hand degerming (Table 12.15).

More recent studies serve to confirm the efficacy and suitability of alcohol-based products as surgical scrubs (Rotter and Koller, 1990, Larson et al., 1990, Paulson, 1994, Hobson et al., 1998). Rotter and Koller (1990) compared the antimicrobial efficacy of three "two-phase" surgical hand disinfection procedures to 60% n-propanol, applied for 5 minutes, which is the reference hand-disinfection procedure used in Austria and Germany. The sequential use of a chlorhexidine gluconate-containing detergent followed by an alcoholic disinfectant reduced the release of resident skin bacteria significantly better than did a sequence of unmedicated soap and alcohol used for the same periods. Paulson compared five surgical hand-scrub preparations (4% CHG brush, 2% CHG solution, providone–iodine brush, *p*-chloro-*m*-xylenol brush, and alcohol-impregnated brush). Only the CHG products demonstrated antimicrobial effectiveness in all three parameters (immediate, persistent, and residual). A comparison also was made between a 5-minute providone–iodine scrub and a 1-minute providone–iodine scrub, followed by alcohol foam (Deshmukh and Kramer, 1998). The total number of colonies was less after the 1-minute scrub with alcohol foam than after the standard 5-minute scrub for both 1-hour and 2-hour groups.

Larson and co-workers investigated both the effect of blood and the effect of a protective foam on the antimicrobial activity of alcohol and other active agents (Larson and Bobo, 1992; Larson et al., 1993). The effect of blood on the efficacy of 70% isopropanol, 0.5% CHG in 70% isopropanol, 7.5% provodine–iodine in a detergent base, 4% CHG in a detergent base, and a nonantimicrobial soap was evaluated in 71 subjects. In the presence of blood, the two alcohol-containing products resulted in significantly greater reductions in the number of colony-forming units (CFUs) than the other products. In the absence of blood, 70% isopropanol was associated with significantly greater reductions; soap resulted in a significantly lower reduction (Larson and Bobo, 1992). The effects of a skin protectant on glove integrity and the efficacy of surgical scrubs with 70% isopropanol, 4% CHG in a detergent base, 7.5% providone–iodine in a detergent base, and a nonantimicrobial soap (control) were determined. No significant differences were found in CFUs on hands with or without protectant immediately after scrubbing or at 2 hours after scrub on gloved or ungloved hands (Larson et al., 1993). The efficacy of alcohol hand rubs with two different kinds of handwashing machines was studied in vivo (Namura et al., 1994). It was concluded that an alcohol-based solution containing an effective antimicrobial detergent preceded by a soap wash is necessary to reduce hand-surface bacteria to a satisfactory degree using these techniques.

Contrary to popular opinion, alcoholic products seem to be quite acceptable to users (Pereira et al., 1997; Boyce, et. al., 1999). Newer formulations containing emollients eliminate the drying effects of alcohol on skin and significantly increase acceptability (Gross et al., 1979; Mitchell and Rawluk, 1984; Mackintosh and Hoffman, 1984; Nystrom, 1984; Larson et al., 1986; Larson et al., 1987; Jones et al., 1986; Rotter et al., 1991; Rotter, 1997; Fendler, 1999). Intermittent use of an alcohol hand gel containing emollients reduced the soap-induced skin irritation of health care personnel and improved their skin condition (cracking, scaling, and redness) and maintained normal skin hydration (Newman and Seitz, 1990). Similar results were found in Finland, where the use of alcoholic preparations containing emollients instead of soap or detergents is recommended to prevent skin problems, especially during winter months (Ojajärvi, 1991).

Several investigators evaluated the use of alcohol-based products for preoperative patient skin preparation. Davies et al. (1978) reported greater than 99% immediate reductions in bacterial counts from skin of the abdomen when using 70% isopropanol, 70% alcoholic chlorhexidine, or providone–iodine. Geelhoed et al. (1983) randomly assigned 173 patients to one of three skin preparation methods: a traditional 5-minute iodophor scrub followed by painting and draping of the skin; the same 5-minute scrub followed by alcohol cleansing (type and percentage of alcohol was unspecified) and application of an iodophor-impregnated drape; or a 1-minute alcohol cleansing followed by application of the antimicrobial drape. The bacterial kill after the 1-minute alcohol cleansing was significantly better than that of the 5-

TABLE 12.15. *Mean log reductions in bacterial counts with various products*

Product	Transient flora; after 30-sec wash*	Normal flora; after surgical scrub†
Plain, unmedicated soap	2.1	<1.0
7.5% providone-iodine liquid soap	2.5	<1.0
4.0% chlorhexidine gluconate liquid soap	2.9	<1.0
70% isopropyl alcohol (vol/vol) + 0.5% chlorhexidine gluconate	3.1	~2.5
70% isopropyl alcohol (vol/vol)	3.3	~2.5
60% *n*-propanol (vol/vol)	3.4	~3.0

*Data from Rotter, 1981.
†Data from Ayliffe et al., 1988.

minute iodophor scrub. Johnston et al. (1987) studied the rate of bacterial recolonization of the skin of the abdomen in 15 volunteers after treatment with chlorhexidine or providone–iodine in 70% alcohol for 3 minutes; 70% isopropanol for 1 minute and application of a plastic adhesive drape; or 70% isopropanol and application of an iodophor-impregnated drape. Although all methods were initially comparable, with bacterial reductions of greater than 99%, recolonization of the site was significantly reduced after 60 minutes at the site prepared with the alcohol and iodophor drape compared with the other methods. Jeng and Severin (1998) investigated the performance of a providone–iodine gel alcohol (5% providone–iodine and 62% ethanol in gel form) as a 30-second, one-time application preoperative skin preparation. The providone–iodine gel alcohol formulation delivered rapid and persistent antimicrobial activity against a broad spectrum of bacteria both in vitro and in vivo and was found to be an effective skin preparation formulation for use in a single-step 30-second application.

Providone–iodine is the most commonly used skin antiseptic for lumbar, thoracic, and cervical epidurals; however, alcohol sometimes is used for single caudal epidurals in children (Burnett et al., 1995). Burnett and colleagues compared the efficacy of 70% isopropanol and 10% providone–iodine for the inhibition of bacterial growth from skin swabs in the caudal area of children and found comparable values for bacterial elimination.

Alcohol is commonly used for skin antisepsis at injection and venipuncture sites. Arata et al. (1997) compared the efficacies of 10% providone–iodine in ethanol and 0.5% CHG in ethanol for antisepsis of injection sites and found no significant differences in bactericidal activity; however, Goldman and Larson (1997) found that isopropanol scrub followed by iodine tincture was more efficacious than providone–iodine as measured by contact plate cultures. For persons who were allergic to iodine, CHG and isopropanol was more efficacious than green soap and isopropanol. Further studies are required to determine whether CHG is superior to providone–iodine or tincture of iodine for this procedure. Maki et al. (1991) demonstrated that significantly fewer catheter infections occurred with 2% aqueous CHG than with 70% isopropanol and 10% providone–iodine (2.3% versus 7.1% and 9.3%, respectively. Alcohol and iodine pads were evaluated for their efficacy of skin "sterilization" prior to venipuncture (Choudhuri et al., 1990). The results indicated that the iodine pads were more effective than alcohol when applied in the manner used in the study. It was unclear, however, whether the difference detected in these microbiologic assays was clinically significant. Butz et al. (1990) reported reductions in colonizing flora following use of 30% ethanol-impregnated towelettes to be comparable to reductions following plain soap. This finding emphasizes the importance of the volume of alcohol to its antimicrobial efficacy.

Effect of Alcohols on Viruses

In contrast to research on the bactericidal effects of alcohols on skin, the development of protocols and studies of the effects of alcohols and other handwashing agents on viruses in vivo began relatively recently. Ansari and co-workers (1989) developed an in vivo protocol for efficacy testing against viruses and used it to evaluate ten antiseptic formulations. Three of the antiseptics and the nonantiseptic soap and tap water were tested against *E. coli*. Ethanol (70%) and isopropanol (70%), alone or with CHG, plus cetrimide reduced the virus titer more than 99%, whereas the reductions for providone–iodine (10%), PCMX (Dettol), and 0.5% CHG in 70% isopropanol ranged from 95% to 97%. The 70% alcohol solutions were significantly more effective than aqueous solutions of CHG. PCMX was much less effective against the virus than against the bacterium. This test protocol also was used to test the effectiveness of ten antiseptic handwashing agents against hepatitis A (HM-175) virus and polio virus type 1 (Sabin) (Mbithi et al., 1993). The 0.3% triclosan soap was most effective against both viruses (92% and 98% reduction, respectively), whereas the reductions by 70% ethanol were 87% and 95%, respectively. Similar reductions of 89% and 97%, respectively, were obtained using a 62% ethanol foam.

Eggers (1990) investigated the effects of alcohols and alcohol-based disinfectants on nonenveloped viruses in vitro and in vivo. Ethanol (80%) in the in vitro suspension test reduced the infectivity of poliovirus 1 by a factor of 10^3 within 2 minutes; however, it was not effective on the skin, with a maximum reduction of sevenfold and only twofold in one case. More favorable results were reported by Nehrkorn and Steinmann (1989), possibly as a result of differences in experimental design. Davies and co-workers (1993) adapted an established protocol for testing bacterial removal from the skin (Ayliffe et al., 1978) for viral removal. This method was used to evaluate the viricidal activity of 70%, 80%, and 90% ethanol; 7.5% providone–iodine; and soap and water against the poliovirus and the *E. coli* bacteriophage. Thorough cleansing followed by use of 90% ethanol was the most effective viricidal treatment. Steinmann et al. (1995) used both whole-hand and fingerpad protocols for testing the viricidal efficacy of a number of commercial handwashing preparations. Using the whole-hand protocol, hand washing with bland soap for 5 min followed by hand rubbing with 80% ethanol resulted in a log reduction of greater than 2; the log reduction by 96.8% ethanol exceeded 3.2.

ABILITY OF ALCOHOLS TO PREVENT INFECTION

The crucial test of any disinfectant is the ability of the substance to prevent infection with the test organism in a susceptible animal. Neufeld and Schiemann (1938)

demonstrated by intraperitoneal injection in mice that a culture of *S. pneumoniae* type 1 was killed by treatment with 80% alcohol. Not all the pneumococci were destroyed when 96% alcohol was used.

Nungester and Kempf (1942) devised a test in which the tail of a mouse was scrubbed with four or five strokes of a swab moistened with a broth culture of the test organism. The tail then was dipped for 2 minutes in a tube containing disinfectant. At the end of this time, a half-inch portion of the tail was cut off and implanted in the peritoneum of the same animal. In the case of *S. pneumoniae* type 1, 100% of the mice died following treatment with either physiologic salt solution, which served as a control, or aqueous thimerosal, 1:1,000. The mortality rate was 69% among the mice when a mixture of 50% alcohol and 10% acetone was used; the mortality rate was 63% when 70% alcohol (by volume) was used. The use of 2% aqueous iodine solution resulted in a mortality rate of only 5%, but when a 2% iodine solution in 95% alcohol was used, the mortality rate was 0%. When using a *hemolytic streptococcus,* none of the animals was protected by the use of the alcohol–acetone mixture.

Using the technique of Nungester and Kempf (1942), Prombo and Tilden (1950) reported a mortality rate of slightly more than 10% when using 70% alcohol by weight and slightly less than 20% when using 70% ethanol by volume. With *S. pyogenes* and 70% alcohol by weight, mortality was 20% in the mice. In preventing infection in mice with *S. pneumoniae,* these researchers found 99% isopropanol as effective as ethanol, 70% by weight, and nearly as effective as ethanol, 70% by volume. It was less effective than ethanol, 70% by weight, in preventing infection in mice with *S. pyogenes.*

Murie and Macpherson (1980) compared postoperative wound infection rates associated with two hand-scrub techniques in the operating room. They alternated each month for 6 months between 0.5% chlorhexidine in 95% methanol and 4% chlorhexidine in detergent. Among 226 patients (117 in one group, 109 in the other), no difference in infection rates was found. Additionally, the alcoholic preparation was five times less expensive, less time consuming, and more acceptable to users. Dorif et al. (1985) compared the use of triple dye versus alcohol (type not specified) for umbilical cord care in neonates and the impact on staphylococcal infections in a newborn nursery over a 1-year period. There was no significant difference in infection rates (0.4% for newborns receiving triple dye and 0.6% for those receiving alcohol treatments). The alcohol-treated infants had fewer cord complications and better healing of umbilical stumps. Butz et al. (1989) evaluated the effect of use of a 60% ethanol hand rinse by day-care providers in 12 centers in reducing transmission of infection among preschool children. Compared with 12 control centers over a 19-month period (8,840 child days), children in 12 test centers had fewer fever days (69 versus 122, $p < 0.001$), vomiting days (11 versus 62, $p < 0.001$), and diarrhea days (77 versus 102, $p = 0.09$). Butz et al. (1990) also found that symptoms of enteric disease (diarrhea and vomiting) were reduced significantly in family day-care homes, where an intervention program was used. The intervention included four components: (1) a handwashing educational program, (2) use of vinyl gloves, (3) use of disposable diaper changing pads, and (4) use of an alcohol-based hand rinse by the day-care provider. Respiratory symptoms did not differ significantly between intervention and control family day-care homes.

A comparative study was carried out to determine the efficacy of 4% CHG in a detergent base and of 60% isopropanol hand rinse (with optional use of a bland soap) in reducing nosocomial infections in intensive care units (ICUs) (Doebbeling et al., 1992). The authors concluded that the CHG product reduced the nosocomial infection rate more effectively than the one using alcohol and soap and attributed the results, at least in part, to better handwashing compliance when the CHG product was used. Goldmann and Larson (1992) stated that this study does not indicate which of the two handwashing agents is superior in the ICU. The hospital personnel used much smaller volumes of alcohol than of CHG, possibly as a result of the smaller amount dispensed per stroke, which could result in insufficient alcohol to wet the hands thoroughly. Goldmann and Larson (1992) emphasized that the debate over handwashing preparations should not overshadow the critical need for hospital personnel to improve their handwashing performance and compliance with barrier precautions. Alcohol preparations kill bacteria rapidly, theoretically permitting briefer washing time. Voss and Widmer (1997) advocated that alcoholic hand disinfection, with its rapid activity, superior efficacy, and minimal time commitment, allows 100% healthcare worker compliance without interfering with the quality of patient care. Alcohol preparations are inexpensive and can be used without a sink when sinks are unavailable or tap water is contaminated—a major advantage in developing countries. "Traditional" alcohol, made from a mixture of maize and manioc, which is fermented and distilled, was used effectively instead of denatured alcohol as an antiseptic for cleansing skin in a rural African setting where supplies are limited (Longombe et al., 1991).

A randomized clinical trial of the effectiveness of an alcoholic solution compared with the standard handwashing procedure in clinical wards and ICUs was carried out in a large public university hospital (Zaragoza et al., 1999). The alcoholic solution resulted in significantly fewer CFUs recovered after the procedure, and overall acceptance was rated as good by 72% of the health care workers. These investigators concluded that the use of alcoholic solutions is effective and safe and deserves more attention, especially in situations in which the compliance rate is hampered by architectural problems or nursing work overload.

ACRONYMS AND ABBREVIATIONS

CDC Centers for Disease Control
CFUs colony-forming units

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